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Monoclonal antibody therapy for the treatment of asthma and chronic obstructive pulmonary disease with eosinophilic inflammation

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ABSTRACT

Eosinophils have been linked with asthma for more than a century, but their role has been unclear. This review discusses the roles of eosinophils in asthma and chronic obstructive pulmonary disease (COPD) and describes therapeutic antibodies that affect eosinophilia. The aims of pharmacologic treatments for pulmonary conditions are to reduce symptoms, slow decline or improve lung function, and reduce the frequency and severity of exacerbations. Inhaled corticosteroids (ICS) are important in managing symptoms and exacerbations in asthma and COPD. However, control with these agents is often suboptimal, especially for patients with severe disease. Recently, new biologics that target eosinophilic inflammation, used as adjunctive therapy to corticosteroids, have proven beneficial and support a pivotal role for eosinophils in the pathology of asthma. Nucala® (mepolizumab; *anti*-interleukin [IL]-5) and Cinquair® (reslizumab; anti-IL-5), the second and third biologics approved, respectively, for the treatment of asthma, exemplifies these new treatment options. Emerging evidence suggests that eosinophils may contribute to exacerbations and possibly to lung function decline for a subset of patients with COPD. Here we describe the pharmacology of therapeutic antibodies inhibiting IL-5 or targeting the IL-5 receptor, as well as other cytokines contributing to eosinophilic inflammation. We discuss their roles as adjuncts to conventional therapeutic approaches, especially ICS therapy, when disease is suboptimally controlled. These agents have achieved a place in the therapeutic armamentarium for asthma and COPD and will deepen our understanding of the pathogenic role of eosinophils.

Abbreviations: ACQ-5, Asthma Control Questionnaire-5; ACQ-6, Asthma Control Questionnaire-6; ADCC, antibody-dependent cell-mediated cytotoxicity; AG, antigen; AHR, airway hyper-responsiveness; AQLQ, Asthma Quality of Life Questionnaire; B, B cell; Bas, basophil; BrO^-, hypobromite; C1q, complement component 1, subcomponent q; CCL, chemokine ligand; CCL11, CC-chemokine ligand 11; CCL13, CC-chemokine ligand 13; CCL17, CC-chemokine ligand 17; CCL22, CC-chemokine ligand 22; CCL24, CC-chemokine ligand 24; CCL26, CC-chemokine ligand 26; CCR, chemokine receptor; CCR3, Chemokine (C-C Motif) Receptor 3; CD, cluster of differentiation protein; CDC, complement-dependent cytotoxicity; COPD, chronic obstructive pulmonary disease; CTL, cytotoxic T lymphocyte; DC, dendritic cell; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; Eos, eosinophil; EPO, eosinophil peroxidase; Fab, fragment antigen-binding; Fc, fragment crystallizable; FcγRIIIa, fragment crystallizable gamma receptor IIIa; FEG, free extracellular granules; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; FGF, fibroblast growth factor; GINA, Global Initiative for Asthma; GM–CSF, granulocyte-macrophage colony-stimulating factor; HER2, human epidermal growth factor receptor 2; HMGB1, high-mobility group box protein 1; H_2O_2, hydrogen peroxide; ICS, inhaled corticosteroids; IFN, interferon; IgE, immunoglobulin E; IgG_1, immunoglobulin G1; IgG_2, immunoglobulin G2; IgG_4, immunoglobulin G4; IgM, immunoglobulin M; IL, interleukin; IL-3, interleukin-3; IL-3R, interleukin-3 receptor; IL-4, interleukin-4; IL-4Rα, interleukin-4 receptor α; IL-5, interleukin-5; IL-5R, interleukin-5 receptor; IL-5Rα, interleukin-5 receptor α; IL-11, interleukin-11; IL-13, interleukin-13; IL-25, interleukin-25; IL-33, interleukin-33; ILC, innate lymphoid cell; ILC2, innate lymphoid cell, type 2; ILR, interleukin receptor; LABA, long-acting β_2 agonist; LT, leukotriene; LTB4, leukotriene B4; LTC4, leukotriene C4; LTD4, leukotriene D4; LTE4, leukotriene E4; mAb, monoclonal antibody; Mac, macrophage; MAC, membrane-attack complex; MBP, major basic protein; MCP-4, monocyte chemoattractant protein 4; MOA, mechanism of action; mRNA, messenger ribonucleic acid; NGF, nerve growth factor; NK, natural killer; O_2, superoxide; OCS, oral corticosteroids; PGD_2, prostaglandin D_2; PDGF, platelet-derived growth factor; PGE_2, prostaglandin E_2; PMN, polymorphonuclear cell; RANTES, regulated on activation, normal T cell expressed and secreted; scFv, single-chain variable fragment; SGRQ, St. George's Respiratory Questionnaire; TARC, thymus- and activation-regulated chemokine; TCR, T-cell receptor; Th2, T-helper 2; scFv, single-chain variable fragment; TGF, transforming growth factor; TGFβ1, transforming growth factor β1; TNF, tumor necrosis factor; TSLP, thymic stromal lymphopoietin; VEGF, vascular endothelial growth factor; VEGF-A, vascular endothelial growth factor A.

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Contents

1. Asthma and chronic obstructive pulmonary disease with eosinophilic inflammation

1.1. Asthma

Asthma is one of the most common chronic diseases, with an estimated 300 million patients afflicted by this disease worldwide. The Global Initiative for Asthma (GINA) 2004 estimated that more than 10% of the population in Australia, Brazil, Canada, New Zealand, Peru, England, and United States had asthma. The prevalence of asthma is expected to increase, and there may be an additional 100 million people with asthma by 2025 (Masoli et al., 2004). A 2012 survey revealed that 25.5 million people in the United States alone, including 6.8 million children younger than 18 years, had a diagnosis of asthma (Centers for Disease Control and Prevention, 2015). Moreover, asthma prevalence has increased from 3.1% in 1980 (Moorman et al., 2007) to 8.3% in 2012 (Centers for Disease Control and Prevention, 2014).

The widespread use of inhaled corticosteroids (ICS) as monotherapy, and particularly as combination therapy with long-acting β_2-agonists (LABA), has been accompanied by a marked reduction in the burden of disease and a substantial reduction in asthma mortality (Chauhan et al., 2015; Chong et al., 2015). However, as with most pharmacotherapies, population data with ICS or ICS–LABA combinations display a Gaussian distribution, with a substantial percentage of patients responding suboptimally or not at all. Furthermore, poor adherence to medication plays an important role in treatment failure (Gamble et al., 2009). By definition, as the requirement for treatment increases, so does the severity of the disease (Global Initiative for Asthma, 2016). In its worst form, severe refractory asthma – which affects 3.6% of patients with asthma – accounts for a disproportionately high burden of suffering and health care costs (Hekking et al., 2015). Suboptimal control in these patients manifests as daytime symptoms, nighttime awakenings, and increased propensity to suffer more frequent and more severe exacerbations, which, in extreme cases, can be fatal.

Increasingly, experts recognize that moderate to severe asthma is a disease encompassing several distinct endotypes characterized by the shared presence of cellular and molecular biomarkers. Linking asthma endotypes with clinical phenotypes has greatly advanced our understanding of asthma pathobiology and the development of novel targeted therapeutics (Anderson, 2008). For more details on this subject, we refer the reader to the reviews by Wenzel, 2012, 2013, 2016; Lötvall et al., 2011. One of the best-characterized endotypes of asthma is the patient population with eosinophilic airway inflammation, which accounts for approximately 40–60% of patients with severe asthma (Wenzel et al., 1999; Douwes et al., 2002; Zhang & Wenzel, 2007; Schleich et al., 2013).

Several important observations in human initially supported the hypothesis that eosinophils play a critical role in the pathogenesis and severity of asthma. An increased eosinophil count is associated with increased asthma severity, frequency of exacerbations and mortality in patients with asthma (Bousquet et al., 1990; Tran et al., 2014; Zeiger et al., 2014). A 1995 report quantified the risk of dying from asthma as 7.4-times greater for patients with eosinophilia than without eosinophilia (Ulrik & Frederiksen, 1995). Several studies have demonstrated that sputum eosinophils increase during exacerbations and that increased numbers of eosinophils in the peripheral blood and airways of patients with asthma correlate with disease severity. Perhaps most importantly, persistent, eosinophilic airway inflammation increases the risk of subsequent exacerbations (Jatakanon et al., 2000; Louis et al., 2000; Di Franco et al., 2003; Miranda et al., 2004; Wenzel, 2005; Scott & Wardlaw, 2006; Zhang & Wenzel, 2007; Malinovschi et al., 2013; Schleich et al., 2014). For example, markers of eosinophilic airway inflammation increase well before the onset of exacerbations induced by corticosteroid withdrawal, and sputum eosinophil number predicts loss of asthma control after corticosteroid reduction or discontinuation (Pizzichini et al., 1999; Jatakanon et al., 2000; Deykin et al., 2005). In addition, activated eosinophils, recognizable by degranulation and upregulation of biochemical effector pathways, are found in the airways of patients who have died of acute severe asthma. Necropsy results identified two distinct pathogenic inflammatory mechanisms of fatal asthma (Restrepo & Peters, 2008). Patients dying suddenly exhibit a neutrophilic airway infiltrate, whereas postmortem examination of patients who exhibit more protracted asthma crises, which precipitate 80–85% of all fatal events, reveals an intense eosinophilic infiltrate (James et al., 2005; Restrepo & Peters, 2008).

Another line of evidence comes from the partial success of eosinophil management strategies with corticosteroids. The English general physician Harry Morrow Brown is credited with making the initial observation that only patients with asthma with eosinophilic inflammation responded to oral steroids (Brown, 1958), which was soon afterward extended to the first effective inhaled steroid, beclomethasone dipropionate (Brown et al., 1972; Brown & Storey, 1973). A more comprehensive study demonstrated that a strategy aimed at managing sputum eosinophils through the controlled use of corticosteroids achieved a greater reduction in the number of severe exacerbations for patients with moderate to severe asthma than a traditional management strategy based on British Thoracic Society guidelines (Green et al., 2002a). More recent studies aimed at controlling sputum eosinophils through adjustments in ICS have provided similar benefits (Chlumský et al., 2006; Jayaram et al., 2006). Although these studies do not prove a cause and effect relationship, as the benefits of controlled corticosteroid management may be mediated through different mechanisms, the findings lend validity to eosinophilic airway inflammation being a surrogate marker for exacerbation frequency. It is unclear how corticosteroids reduce eosinophilic inflammation. Some studies suggest that corticosteroids reduce IL-5 receptor (IL-5R) expression on eosinophils, rendering them less responsive to IL-5. Other studies propose that corticosteroids increase eosinophil apoptosis and stimulate phagocytic elimination of apoptotic eosinophils (Her et al., 1991; Meagher et al., 1996; Liu et al., 1999).

These initial discoveries in humans of a pathogenic role for eosinophils in asthma were confirmed by application of mouse genetics. Two

strains of eosinophil-deficient mice respond to pulmonary allergen provocation with reduced collagen deposition, airway smooth muscle accumulation, airway hyper-responsiveness (AHR), and mucus hypersecretion (Humbles et al., 2004; Lee et al., 2004). Mice deficient in IL-5 or IL-5 receptor-α (IL-5Rα) respond similarly, highlighting the roles of IL-5 and IL-5Rα as critical pathways for eosinophil development, differentiation, expansion, and activation (Foster et al., 1996; Tanaka et al., 2004). These findings were both strain- and model-dependent.

Finally, the most recent and conclusive evidence for a pathogenic role of eosinophils in asthma comes from therapeutic antibodies that either antagonize IL-5 (mepolizumab and reslizumab) or have been engineered to eliminate eosinophils via antibody-dependent cell-mediated cytotoxicity (ADCC; benralizumab) in patients with inadequately controlled asthma with eosinophilic inflammation despite the use of medium- to high-dosage ICS and β-agonists. All three antibodies reduce eosinophilic inflammation to various degrees, which has been demonstrated to correlate with reduction in acute exacerbations and improvement in lung function and asthma control in some studies. These therapies are discussed in Section 6 (Haldar et al., 2009; Nair et al., 2009; Castro et al., 2011, 2014, 2015; Pavord et al., 2012; Bel et al., 2014; Ortega et al., 2014; Nowak et al., 2015).

1.2. Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) describes a group of progressive, debilitating respiratory conditions, including emphysema and chronic bronchitis, characterized by difficulty in breathing, airflow limitation, cough, and other symptoms. In contrast with asthma, irreversible airflow obstruction, caused by structural damage to the elastic microstructure of lung parenchyma and small airway remodeling, is a key COPD feature. COPD affects approximately 380 million people worldwide, it is the fourth greatest cause of mortality globally and predicted to become the third by 2020 (Adeloye et al., 2015). COPD is overwhelmingly associated with a history of cigarette or pipe smoking. However, with increasing exposure to air pollution and poor indoor air quality – compounded by other insults that retard lung growth in utero and in the early years of life – as much as 40% of the population-attributable risk for COPD is now thought to be independent of cigarette smoke exposure (World Health Organization, 2014a, 2014b; Lange et al., 2015).

COPD is a disease with predominantly neutrophilic inflammation of the airways, and disease pathogenesis involves neutrophils, together with $CD8^+$ T cells and macrophages. Nevertheless, approximately 20–40% of patients with COPD have elevated eosinophils in their airways, some despite treatment with ICS (Saha & Brightling, 2006). The relationship between eosinophilic inflammation and disease severity in COPD is unclear, with some cross-sectional studies reporting negative correlations between eosinophil numbers and FEV_1 (Balzano et al., 1999; Lams et al., 2000), while others have reported no relationship between eosinophilia and COPD disease severity (Hogg et al., 2004). However, patients with COPD who have elevated blood or sputum eosinophil counts were more responsive to corticosteroid therapy (Saetta et al., 1994; Zhu et al., 2001; Saha & Brightling, 2006). Treatment with oral corticosteroids may reduce the numbers of circulating and sputum eosinophils in these patients and reduced the frequency of severe exacerbations (defined as worsening of respiratory symptoms resulting in hospital admission) by approximately 62% (Siva et al., 2007). This phenomenon may account for the beneficial effects of corticosteroids for a subgroup of patients in a disease that is otherwise substantially resistant to steroid therapy. Confirmation of this hypothesis will require the use of therapeutics that specifically target eosinophils. Given that ICS therapies are linked with increased dosage-proportional risk of pneumonia in COPD, there is even greater interest in non-steroid eosinophil-directed treatment. In a clinical Phase IIa study of 101 patients with COPD and elevated sputum eosinophil counts, benralizumab, a humanized, afucosylated, anti-IL-5Rα monoclonal antibody, depleted sputum and blood eosinophils and basophils by enhanced antibody-dependent cell-mediated cytotoxicity. For these patients, benralizumab had no effect on exacerbations but significantly improved FEV_1 in comparison with placebo (Brightling et al., 2014). Phase III clinical studies are currently underway with benralizumab (NCT02138916, NCT02155660) and mepolizumab (anti-IL-5; NCT02105948, NCT02105961), addressing safety and efficacy in these patients. In addition, one Phase III study with mepolizumab has recently completed (NCT01463644).

2. The eosinophilic granulocyte

2.1. Eosinopoiesis

Eosinophils are terminally differentiated granulocytes with the properties of cytotoxic effector cells. Eosinophils develop in bone marrow derived from $CD34^+$ pluripotent hematopoietic stem cells. Human eosinophil lineage-committed progenitor cells express the interleukin-5 receptor (IL-5R) and are direct descendants of IL-5Rα$^+$ myeloid progenitors, distinct from granulocyte–macrophage progenitors that give rise to neutrophils and basophils. Eosinophil progenitors undergo differentiation in bone marrow where they are exposed to IL-3, granulocyte-macrophage colony-stimulating factor (GM–CSF), and IL-5. Of these factors, IL-5 plays a leading role in promoting expansion of the eosinophil lineage from committed progenitors in the bone marrow (Lopez et al., 1986, 1988; Shalit et al., 1995; Mori et al., 2009).

Eosinophils emerge fully differentiated into the blood stream, where they have a relatively short half-life of approximately 18 h owing to their rapid migration into tissues. In healthy individuals, eosinophils make up approximately 1–5% of circulating leukocytes and populate peripheral mucosal tissues, such as the gastrointestinal tract, female reproductive organs, and lymphoid tissues. Eosinophils also develop from $CD34^+$ progenitor cells outside the bone marrow, notably in lung tissue. Mobilization of $CD34^+$ progenitors from bone marrow to the lungs occurs in mouse models of allergic airway inflammation (Southam et al., 2005) and in patients with asthma (Robinson et al., 1999a; reviewed by Gauvreau & Denburg, 2005). Progenitors in the lungs may differentiate into mature eosinophils depending on the local tissue environment and under the influence of soluble factors such as IL-5, IL-3, GM–CSF, thymic stromal lymphopoietin (TSLP), IL-33, leukotrienes and CC chemokine ligand 11 (CCL11; eotaxin-1) (Dorman et al., 2004a, 2004b; Allakhverdi et al., 2009; Smith et al., 2015). Anti-IL-5 treatment in humans with mepolizumab induced only partial maturational arrest of the eosinophil lineage in the bone marrow, a reduction in airway $CD34^+$/IL-5Rα mRNA$^+$ cell numbers (Menzies-Gow et al., 2003), and a partial reduction of mature tissue eosinophils in patients with asthma (Flood-Page et al., 2003a; Haldar et al., 2009). These observations demonstrate that, in humans, factors other than IL-5 contribute to the differentiation and survival of eosinophils in the bone marrow and pulmonary mucosa. Thus, inhibiting IL-5 alone may not be adequate to eliminate tissue eosinophils rapidly.

2.2. Eosinophil granules

When first described by Paul Ehrlich in 1878, the eosinophil was so named because of the exuberant staining of its specific secondary granules with the red dye eosin (Ehrlich, 1878). Human eosinophil-specific secondary granules are a store of diverse, preformed cationic proteins. They contain four major proteins: eosinophil peroxidase (EPO), major basic protein (MBP), and the ribonucleases, eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN), but also store several cytokines, enzymes, and growth factors. Other prominent features of eosinophils include primary granules containing Charcot–Leyden crystal protein (galectin 10, eosinophil lysophospholipase) and lipid bodies, which are sites of synthesis of cysteinyl leukotrienes, eoxins, thromboxane, and prostaglandins.

Eosinophils release their granule products in three different ways: cytolysis, exocytosis, and piecemeal degranulation. When eosinophils die by lysis, they liberate intact, membrane-bound free extracellular granules (FEGs) (Neves & Weller, 2009; Lacy & Moqbel, 2012). Receptor-mediated activation of these FEGs results in secretion of selected granule-derived proteins. Although FEGs are present in human disease, their pathological significance is the subject of active debate (see discussion in Section 3 below; Persson & Uller, 2014). Eosinophil exocytosis, through which intracellular granules fuse with the plasma membrane to release their entire contents, tends to occur principally in response to parasites, on the surfaces of large, multicellular helminths.

Eosinophils can also selectively discharge distinct mediators, even though these are actually co-stored in the same secretory granules, by a process termed piecemeal degranulation, first reported by Ann Dvorak (Dvorak et al., 1991; Melo & Weller, 2010). In this process, distinct from classical exocytosis, secretory granules maintain their integrity because fusion events among granules and between granules and the plasma membrane do not occur. Piecemeal degranulation is the most commonly observed physiologic form of eosinophil degranulation. The identification of these granule products in blood offers the possibly of developing biomarker panels that may reflect eosinophil number and activity state.

2.3. Host defense

Phylogenetically, eosinophils are ancient and predate the development of T cells. The T-cell/eosinophil nexus likely arose under selection pressure from helminthic parasites. Reports of the antiparasitic features of eosinophils occur as far back as 1939. Helminths are parasitic worms that typically induce a T-helper 2 (Th2) immune response in their hosts, promoting immunoglobulin E (IgE) responses and eosinophilia. Peripheral and tissue eosinophilia and increased blood concentrations of eosinophil-derived granule proteins occur in helminth-infected individuals. In vitro, eosinophils can attach to the surface of helminth larvae (Shin et al., 2001), release damaging granule contents (Hamann et al., 1990), and kill worms in antibody- and complement-dependent fashion (Butterworth et al., 1975; Rainbird et al., 1998; Shin et al., 2001). However, in vivo, the picture is more complicated. For example, in humans, the 434CC polymorphism, resulting in a less cytotoxic form of the eosinophil granule protein ECP, is more common among Ugandans, who live in a region endemic for infection by the parasitic helminth *Schistosoma mansoni* (Eriksson et al., 2007). By contrast, the 434CC genotype is quite rare in Sudan, where *S. mansoni* is not endemic, suggesting no selective disadvantage for individuals with the 434CC polymorphism to mount *anti*-schistosomal host defense (Eriksson et al., 2007). Interestingly, carriers of the 434CC genotype develop substantially less liver fibrosis secondary to *S. mansoni* infection (Eriksson et al., 2007), pointing to the potential of ECP to cause tissue injury in vivo. This study and other studies in humans suggest the importance of inducing an effective antihelminthic eosinophil response while minimizing collateral damage to affected tissues. The questionable importance of eosinophils in the host response to parasites has been corroborated by the variable outcomes of eosinophil suppression in preclinical animal models of parasite infection (Sher et al., 1990; Herndon & Kayes, 1992; Shin et al., 1997; Betts & Else, 1999). Overall, the role of eosinophils in parasite infections remains unclear and may be parasite-dependent.

Eosinophils have also been implicated in the host response to respiratory viral infections in preclinical models of respiratory syncytial virus (Domachowske et al., 1998; Phipps et al., 2007) and parainfluenza infections (Adamko et al., 1999). Indeed, eosinophilia has been reported in experimental human rhinovirus-16 infection studies in healthy individuals and patients with asthma, and human respiratory viruses (most prominently rhinoviruses) are among the more common causes of asthma exacerbations (Fraenkel et al., 1995; Grünberg et al., 1997; Jackson et al., 2014). The fact that respiratory viruses are a common cause of exacerbations in asthma, combined with the observation that biologics targeting the IL-5 and IL-5Rα pathways lead to decreased eosinophilic inflammation and decreased frequency of exacerbations, to varying degrees, argues for a dispensable role of eosinophils in viral host defense under such circumstances.

2.4. Drivers of eosinophil recruitment and activation

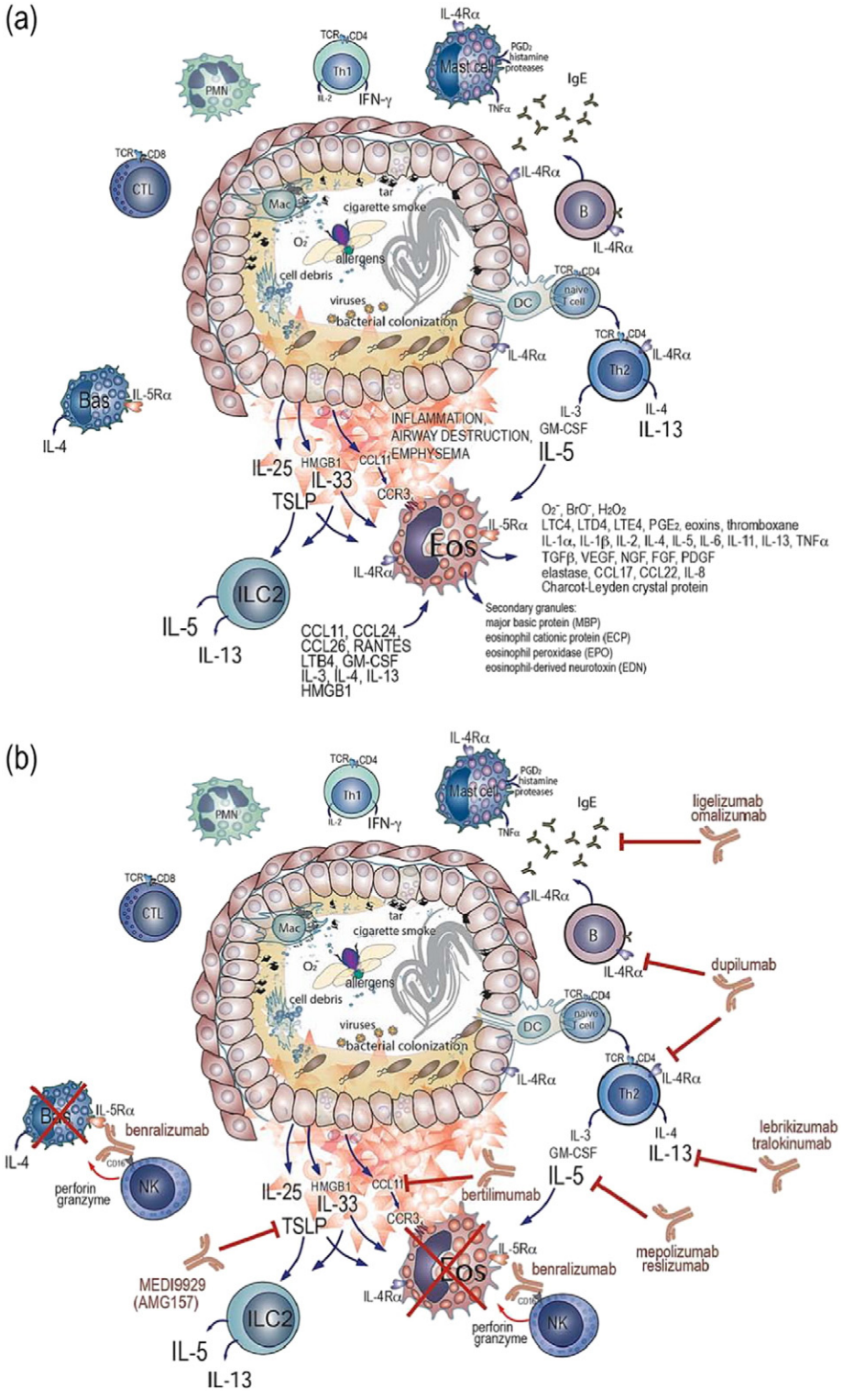
Although IL-5 is the primary regulator of eosinophil migration into the circulation, additional cytokines and chemokines contribute to directing eosinophil migration into inflamed tissues. Nevertheless, IL-5 does play a particularly important role in eosinophil recruitment. It synergizes with other Th2 cytokines, IL-4 and IL-13, and with the eosinophil chemo-attractants, CCL11, CCL24 and CCL26, to promote eosinophil activation and recruitment into tissues in acute inflammatory responses (Collins et al., 1995; Mould et al., 1997; Foster et al., 2001; Pease & Williams, 2001). However, other mediators also play a significant role in recruiting eosinophils to sites of inflammation. Among these are the cytokines TSLP, IL-25, and IL-33, which structural cells release in response to various environmental insults. TSLP, IL-25, and IL-33 are distinct cytokines with significant overlapping biology and a common function in promoting Th2 responses. In the airways, these three early cytokines target a range of similar cell types; and all promote eosinophilic inflammation, at least in part by inducing IL-5 production and by amplifying Th2 responses (Allakhverdi et al., 2007; Neill et al., 2010; Mjosberg et al., 2011; Ikutani et al., 2012; Kim et al., 2013a,

Fig. 1. (a) Eosinophilic inflammation in asthma and COPD. Allergens, pathogens, cigarette smoke, toxins, and other particulate matter accumulate in the airway lumen and result in the activation and injury of airway epithelial cells. Epithelial cells release damage-associated molecular pattern molecules (HMGB1) and innate cytokines (IL-33, IL-25, TSLP) that initiate or amplify airway inflammation. Depending on the quality and combination of stimuli, a variety of innate immune cells are recruited and activated in the lung (e.g., eosinophils, mast cells, dendritic cells, macrophages, ILC2, basophils, neutrophils). Consequently, adaptive immune responses are initiated, exemplified by the accumulation and activation of T and B cells. In combination, the innate and adaptive immune cells are the source of mediators that amplify inflammation in many different ways, including reactive oxygen species (O_2^-, BrO^-, H_2O_2), lipid mediators (LTB4, LTC4, LTD4, LTE4, eoxins, thromboxane), cytokines (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-11, IL-13, interferon-γ, GM–CSF, TNFα), growth and differentiation factors (TGFβ, VEGF, NGF, FGF, PDGF), proteases (elastase), chemokines (CCL11, CCL17, CCL22, CCL24, CCL26, RANTES, IL-8), Charcot-Leyden crystal protein, prostaglandins (PGD_2, PGE_2), immunoglobulins (IgE), and histamine. Together, these cells and their mediators also drive airway hyperresponsiveness and constriction, airway remodeling, mucus hypersecretion and epithelial hyperplasia, all associated with clinical sequelae of asthma and COPD. Eosinophils are a key inflammatory cell whose persistence is directly regulated by chemokines (CCL11, CCL24, CCL26, RANTES), lipids (LTB4), cytokines (GM–CSF, IL-3, IL-4, IL-5, IL-13, IL-25, IL-33, TSLP), and damage-associated molecular pattern molecules (HMGB1). Eosinophils are a rich source of a large number of mediators, including basic granule proteins (MBP, ECP, EPO, EDN) that are toxic and can injure neighboring tissues. (b) Therapeutic monoclonal antibodies for asthma and COPD. Monoclonal antibodies in clinical development that have been demonstrated to affect eosinophilic inflammation in asthma or COPD are indicated. The red T-shaped symbols indicate the molecular target of the respective antibody, which is shown in brown, and its antagonistic activity against the target. Benralizumab is the only ADCC-enhanced antibody resulting in target cell apoptosis, symbolized by the red X.Abbreviations: ADCC (antibody-dependent cell-mediated cytotoxicity), B (B cell), Bas (basophil), BrO^- (hypobromite), CD (cluster of differentiation protein), CCL (chemokine ligand), CCR (chemokine receptor), COPD (chronic obstructive pulmonary disease), CTL, (cytotoxic T lymphocyte), DC (dendritic cell), ECP (eosinophil cationic protein), EDN (eosinophil-derived neurotoxin), Eos (eosinophil), EPO (eosinophil peroxidase), FGF (fibroblast growth factor), GM–CSF (granulocyte-macrophage colony-stimulating factor), H_2O_2 (hydrogen peroxide), HMGB1 (high-mobility group box 1 protein), IFN (interferon), IgE (immunoglobulin E), IL (interleukin), ILC (innate lymphoid cell), IL-4Rα (interleukin-4 receptor α), IL-5Rα (interleukin-5 receptor α), LTB4 (leukotriene B4), LTC4 (leukotriene C4), LTD4 (leukotriene D4), LTE4 (leukotriene E4), Mac (macrophage), MBP (major basic protein), NGF (nerve growth factor), NK (natural killer cell), O_2^- (superoxide), PDGF (platelet-derived growth factor), PGD_2 (prostaglandin D_2), PGE_2 (prostaglandin E_2), PMN (polymorphonuclear cell = neutrophil), RANTES (regulated on activation, normal T cell expressed and secreted protein), TCR (T-cell receptor), Th (T-helper cell), TGF (transforming growth factor), TNF (tumor necrosis factor), TSLP (thymic stromal lymphopoietin), VEGF (vascular endothelial growth factor).

2013b). In addition, TSLP and IL-33 can act directly on eosinophils. TSLP delays apoptosis of eosinophils (Wong et al., 2010), whereas IL-33 potently activates murine eosinophils and increases the survival of human eosinophils (Cherry et al., 2008; Wen et al., 2013).

In addition, growth factors such as GM–CSF (Woolley et al., 1995; Simon et al., 1997; Wang et al., 2007; Griseri et al., 2015) and molecules with an alarmin function such as high-mobility group protein 1 (HMGB1) can also recruit and activate eosinophils, but they appear to act independently of IL-5. Further research in this area may help better understand the mechanisms by which eosinophils are activated, in particular in response to damage-associated molecular pattern molecules such as HMGB1 (Lotfi et al., 2007; Rosenberg et al., 2013). These molecules may play a significant role in eosinophil recruitment associated with nonpulmonary conditions such as myalgias and myopathies.

Fig. 1 provides the main structural and hematopoietic cells and their secreted factors that orchestrate the recruitment, differentiation, activation, and maintenance of eosinophils in the airways of asthma and COPD patients. For additional reading about the biology of eosinophils in host



defense and disease, we would like to direct the reader to two excellent and comprehensive reviews (Rothenberg & Hogan, 2006; Rosenberg et al., 2013).

3. Pathologic functions of eosinophils

Pathologic conditions of the lung can lead to the increase in eosinophil production in the bone marrow and the migration of these cells along chemokine or cytokine gradients into the lung, where they contribute to the manifestation of clinical phenotypes. Eosinophils can contribute to pulmonary inflammation through several mechanisms. A key action is through the release of cytotoxic granule-associated basic proteins, reactive oxygen species, and lipid mediators, which collectively can damage surrounding cells and induce AHR and mucus hypersecretion. Bronchial biopsies from patients with asthma indicate an association between eosinophil numbers and epithelial damage (Wilson et al., 2013), and eosinophil granule proteins are cytotoxic toward epithelial cells in vitro. In addition, eosinophils are an important source of mediators of airway narrowing, such as leukotriene *C*4, and eosinophil degranulation has been linked to AHR in animal models and humans.

Significant lysis of eosinophils can occur in asthmatic airways, releasing free eosinophilic granules into bronchial tissue. The evidence for eosinophil lysis in bronchial tissues and its part in damaging the airways was recently reviewed (Persson & Uller, 2014). Transmission electron micrographs of asthmatic airway tissues demonstrate "spilling" of FEGs from lytic eosinophils and immunostaining for toxic granule proteins shows FEGs in numerous asthmatic bronchi. In contrast, there are few signs of eosinophil apoptosis in these tissues (Uller et al., 2006). FEGs occur strikingly in some instances of lethal asthma, but few intact eosinophils are present (Filley et al., 1982) and FEGs are clearly increased in sputum at severe exacerbations (Pizzichini et al., 1997). In addition, studies have suggested an association between the presence of FEGs, rather than intact eosinophils, and epithelial disruption in asthmatic airways (Filley et al., 1982; Woolley et al., 1995; Fattahi et al., 2013; Volbeda et al., 2013). These studies suggest that therapeutics directed at suppressing eosinophilic inflammation should do so by mechanisms that avoid eosinophil lysis and degranulation.

Eosinophils also have an important role in promoting both the recruitment and activation of Th2 cells in the lungs, and this process potentially occurs through multiple mechanisms. Eosinophils release preformed cytokines, such as IL-4 and IL-13, that amplify Th2 responses (Spencer et al., 2009). They also release Th2-type chemoattractants, such as CCL17 and CCL22, which promote recruitment of Th2 T cells (Jacobsen et al., 2008). Eosinophils may also have an antigen-presenting function. Although they are not "professional" antigen-presenting cells, eosinophils do express cell surface molecules needed for antigen presentation. They can process antigens and stimulate T cells in an antigen-specific manner, resulting in T-cell proliferation and cytokine release (Wang et al., 2007).

Eosinophils can interact directly or indirectly with other innate cell populations to maintain and enhance a Th2 environment. These interactions currently are not very well-defined, but they may also contribute to tissue remodeling processes such as fibrosis. Eosinophilic infiltrates and eosinophil granule proteins are present in fibrotic tissues from many different organs, including the lungs, and are associated with rapid disease progression in patients with idiopathic pulmonary fibrosis (Fujimoto et al., 1995; Birring et al., 2005). In asthma, eosinophils could contribute to subepithelial fibrosis through generation of profibrotic mediators such as transforming growth factor beta 1 (TGFβ1) and IL-11; and several reports claim that the majority of TGFβ1 mRNA-positive cells in bronchial biopsies of patients with severe asthma are eosinophils (Minshall et al., 1997; Vignola et al., 1997; Flood-Page et al., 2003b). Human eosinophils cultured with fibroblasts augment the contraction of 3-dimensional collagen matrices (Zagai et al., 2004), and crosstalk between mast cells and eosinophils may influence fibroblast behavior in allergic inflammation (Elishmereni et al., 2011). Functional evidence also comes from in vivo studies in eosinophil-deficient mice that demonstrated decreased airway extracellular matrix deposition following allergen challenge (Humbles et al., 2004). Observations in patients with asthma corroborate these findings. Eosinophil suppression via anti-IL-5 treatment (mepolizumab) in patients with mild atopic asthma resulted in reduced deposition of extracellular matrix proteins in the bronchial subepithelial basement membrane (Flood-Page et al., 2003b) and, in a subsequent study in patients with severe eosinophilic asthma, in reduced airway-wall thickness and total wall area (Haldar et al., 2009). Whether and how eosinophil-mediated fibrosis of the airways contributes to the pathogenesis of asthma or other diseases characterized by eosinophilic inflammation remains to be determined.

Fig. 1 depicts some of the main products of activated eosinophils in the airways, which contribute to airway damage and which are associated with pathophysiologic alterations and inflammation.

4. Clinical need in asthma and chronic obstructive pulmonary disease with eosinophilic inflammation

Most asthma guidelines include a stepwise approach to treatment whereby control is achieved by stepping up treatment as necessary. Inhaled corticosteroids (ICS), first introduced in the early 1970s, are the cornerstone of treatment for asthma, improving lung function, and reducing symptoms and exacerbations (Rodrigo, 2006).

Early studies of ICS did not focus particularly on eosinophilic asthmatics, although several studies suggested that the presence of persistent airway eosinophilic inflammation seemed to be a good predictor of short-term response to ICS. Many studies have indicated that continual treatment with ICS rapidly reduces sputum eosinophilia (Fahy & Boushey, 1998; Jatakanon et al., 1998; Meijer et al., 1999; van Rensen et al., 1999; Aldridge et al., 2000).

Early studies of patients with eosinophilic and non-eosinophilic asthma investigating short-term ICS treatment were small and not placebo-controlled (Lim et al., 1999; Pavord et al., 1999; Green et al., 2002b; Jang et al., 2005; Bacci et al., 2006). The first randomized controlled trial compared 8 weeks' treatment with ICS in patients with eosinophilic vs. non-eosinophilic asthma and demonstrated better improvement in airway hyper-responsiveness and health-related quality of life for eosinophilic patients (Berry et al., 2007). Subsequently, Woodruff et al. demonstrated that lung function improvements with ICS were restricted to Th2-high asthma patients, a phenotype that included patients with elevated blood and airway eosinophilia (Woodruff et al., 2009). In a larger study, McGrath et al. found that low-dosage ICS (4–6 weeks of treatment) achieved good asthma control for a greater percentage of mild to moderate asthma patients who were eosinophilic (sputum eosinophils > 2%) than non-eosinophilic asthma patients (McGrath et al., 2012).

Several studies have investigated long-term treatment with ICS therapy for eosinophilic asthma patients. Three randomized controlled trials of patients with moderate to severe asthma have demonstrated that normalizing airway eosinophil numbers by titrating ICS for approximately 1 year significantly reduced the frequency and severity of asthma exacerbations when compared with just following treatment guidelines (Green et al., 2002a; Chlumský et al., 2006; Jayaram et al., 2006). However, there is a paucity of large, long-term studies directly comparing the benefits of ICS treatment on asthma control for eosinophilic versus non-eosinophilic patients.

Despite the effectiveness of ICS in treating exacerbations for many patients with eosinophilic asthma, a subset of patients have persistent tissue eosinophilia despite receiving high-dosage ICS and oral corticosteroids (Synek et al., 1996; Jatakanon et al., 1999; Louis et al., 2000; ten Brinke et al., 2001; Miranda et al., 2004). Approximately one-half to two-thirds of patients with difficult-to-treat asthma are estimated to also have persistent tissue eosinophilia despite receiving high-dose ICS or oral

corticosteroids (Wenzel et al., 1999; Wenzel, 2005; van Veen et al., 2009), suggesting the presence of a distinct steroid-resistant eosinophilic phenotype (Wenzel et al., 1999). Yet, the molecular mechanisms contributing to "corticosteroid-resistant" eosinophilic inflammation are unclear.

Persistent corticosteroid usage has other limitations and, in particular, is associated with significant adverse effects (Elixhauser & Owens, 2007; Manson et al., 2009; Sarnes et al., 2011). Long-term treatment with high-dosage ICS is associated with systemic adverse effects (Pandya et al., 2014), including reduced bone mineral density (Wong et al., 2000; Israel et al., 2001; Kelly, 2003; Monadi et al., 2015) associated with osteoporotic fractures. Oral corticosteroids cause significantly greater morbidity, and high dosages of oral corticosteroids needed to partially control severe asthma in some patients frequently contribute to significant adverse effects, such as coronary heart disease, psychiatric comorbidities, osteoporosis, diabetes and pneumonia (Iribarren et al., 2012; Amelink et al., 2014; Zazzali et al., 2015).

Nonadherence to ICS is a significant reason for poor asthma control, asthma-related emergency visits and hospitalizations, and increased oral steroid use (Williams et al., 2004; Gamble et al., 2009; Murphy et al., 2012). Even for refractory patients with persistent symptoms, poor long-term adherence to ICS regimens is still an issue (Gamble et al., 2009; Wu et al., 2015). In addition, nonadherence to short-term corticosteroid therapy (inhaled or systemic) is surprisingly common for adult asthmatics discharged from the emergency department or hospital after initial therapy for acute asthma and is a key factor associated with relapse (Krishnan et al., 2004; Williams et al., 2004). Nonadherence to short-term treatment with oral corticosteroids following discharge from the emergency department has been associated with poor outcomes for some pediatric patients with persistent asthma (Cooper & Hickson, 2001; Butler & Cooper, 2004; Williams et al., 2013).

Despite the advent of newer and effective therapies, many patients with COPD continue to have exacerbations and deterioration of lung function and quality of life. Hence, a substantial need remains for improving lung function, achieving better symptom control, reducing exacerbations, slowing disease progression, and improving life expectancy (Calverley, 2008). The use of inhaled corticosteroids in combination with other medications, such as long-acting beta agonists, has been associated with an improvement in exacerbation rates and health-related quality of life in patients with COPD (Global Initiative for Chronic Obstructive Lung Disease, 2016). It is not clear which patients benefit the most from inhaled corticosteroids, but it is clear that the response is heterogeneous. In a meta-analysis of 12 randomized controlled trials (comprising 1331 patients with acute exacerbations of COPD treated with systemic corticosteroids), pooled analysis demonstrated a beneficial effect of treatment. However, when analyzed by subgroups, treatment benefit was observed only for non-critically ill patients (those not admitted to intensive care), and no effect on patient mortality was observed for any group (Abroug et al., 2014). Changes in sputum cell counts have also yielded heterogeneous results, with numbers of neutrophils, eosinophils, and macrophages demonstrated to be unaltered or reduced following corticosteroid therapy (Saha & Brightling, 2006). More specific and effective therapeutic agents directed at eosinophilic inflammation may provide additional options for some patients who do not appear to respond adequately to corticosteroid therapy.

Thus, the clinical need in patient populations with asthma and COPD with eosinophilic inflammation presents an ideal opportunity for the development of biologic long-acting therapies that more completely and selectively suppress airway eosinophilic inflammation.

5. Potential of therapeutic antibodies

During the last 30 years, therapeutic antibody development has blossomed, with the realization that these agents offer high specificity and affinity for molecular targets that are frequently intractable to small-molecule approaches. This rapid progress has seen biologics become a mainstay of therapeutic options for patients with autoimmune and inflammatory diseases. For example, sales of the tumor necrosis factor antagonist adalimumab reached $10 billion, making it the biggest-selling drug for that year (King, 2014).

Efficacy of early murine or human/mouse chimeric antibodies was limited by development of immunogenicity in patients. Thus, most antibodies now entering clinical trials have been humanized or are fully human antibodies, which generally yield much reduced immunogenicity. Many therapeutic antibodies work by binding and neutralizing their molecular targets through their variable Fab domains, while their Fc domains are engineered to be "immunologically silent" (Fig. 2a–c). Examples discussed in this review include *anti*-IgE (omalizumab, ligelizumab), anti-IL-5 (mepolizumab, reslizumab), anti-IL-13 (tralokinumab, lebrikizumab), anti-TSLP (AMG157/MEDI-9929), anti-CCL11 (bertilimumab), and anti-IL-4Rα (dupilumab; see Fig. 1b and Table 1). Improvements in binding affinity are routinely achieved through a range of optimization strategies generating, in some cases, monoclonal antibodies with exquisitely high affinities for their target antigens (Finch et al., 2011; Lowe et al., 2011). Alternatively, a therapeutic antibody can highly selectively bind an antigen on the surface of target cells while its Fc domain can actively engage complement or immune cells, resulting in the selective elimination of these target cells via complement-dependent cytotoxicity (CDC) or ADCC, respectively (Fig. 2d,e and Table 1). The anti-IL-5Rα antibody benralizumab is an example of an ADCC-enhancing antibody that induces cell death of IL-5Rα-expressing eosinophils and basophils.

Therapeutic antibodies typically have long serum half-lives, which allow for infrequent administration, every other week or less frequently (Roopenian & Akilesh, 2007; Finch et al., 2011; Robbie et al., 2013; Souders et al., 2015), an advantage in situations in which the targeted patient population adheres poorly to standard of care. Systemic administration can intravenous or subcutaneous, the latter potentially allowing the convenience of self-administration for patients with chronic diseases. Inhaled delivery of therapeutic antibodies is particularly relevant for pulmonary indications to optimize drug delivery to the luminal surfaces of the airways or to avoid systemic safety issues. However, this route of administration presents challenges, such as residence time of therapeutic antibodies in the lung and delivery of antibodies across the mucus barrier and to the lower airways. Although several inhaled therapeutic proteins are either in late-stage clinical development (e.g., α-1 *anti*-trypsin [AAT; Kamada]) or approved and marketed (e.g., DNase for cystic fibrosis [dornase alfa; Pulmozyme®; Genentech]), there are not yet any marketed, inhaled therapeutic antibodies.

The high potency and specificity of therapeutic antibodies for particular molecular targets is a major advantage in maximizing their clinical efficacy and limiting their adverse effects. However, they are frequently reserved for patients with more severe manifestations of diseases who consume considerable health care resources. Improving the understanding of the different molecular mechanisms underpinning patient heterogeneity in a disease such as asthma allows the identification of key molecular targets for different patient subpopulations and the tailoring of therapeutic antibody treatments to those patients most likely to respond, thus maximizing the value of each therapy.

Polypharmacy is often required to optimize therapy for complex indications. Therapeutic antibodies are often an adjunct to current standard of care, providing additional benefit for patients whose disease is insufficiently controlled with approved medications, which could apply to patients with poorly controlled asthma and COPD. Combining therapeutic antibody treatments may be a viable option. Co-administration of two separate therapeutic antibodies in the clinical setting allows dosing flexibility, but it may be an advantage to combine therapies in a single vial or even a single molecule (bispecific antibodies, Fig. 2g). The emergence of alternative formats and improvements in generating and producing bispecific antibodies have driven a resurgence of interest in this field (Kontermann & Brinkmann, 2015; Spiess et al., 2015).

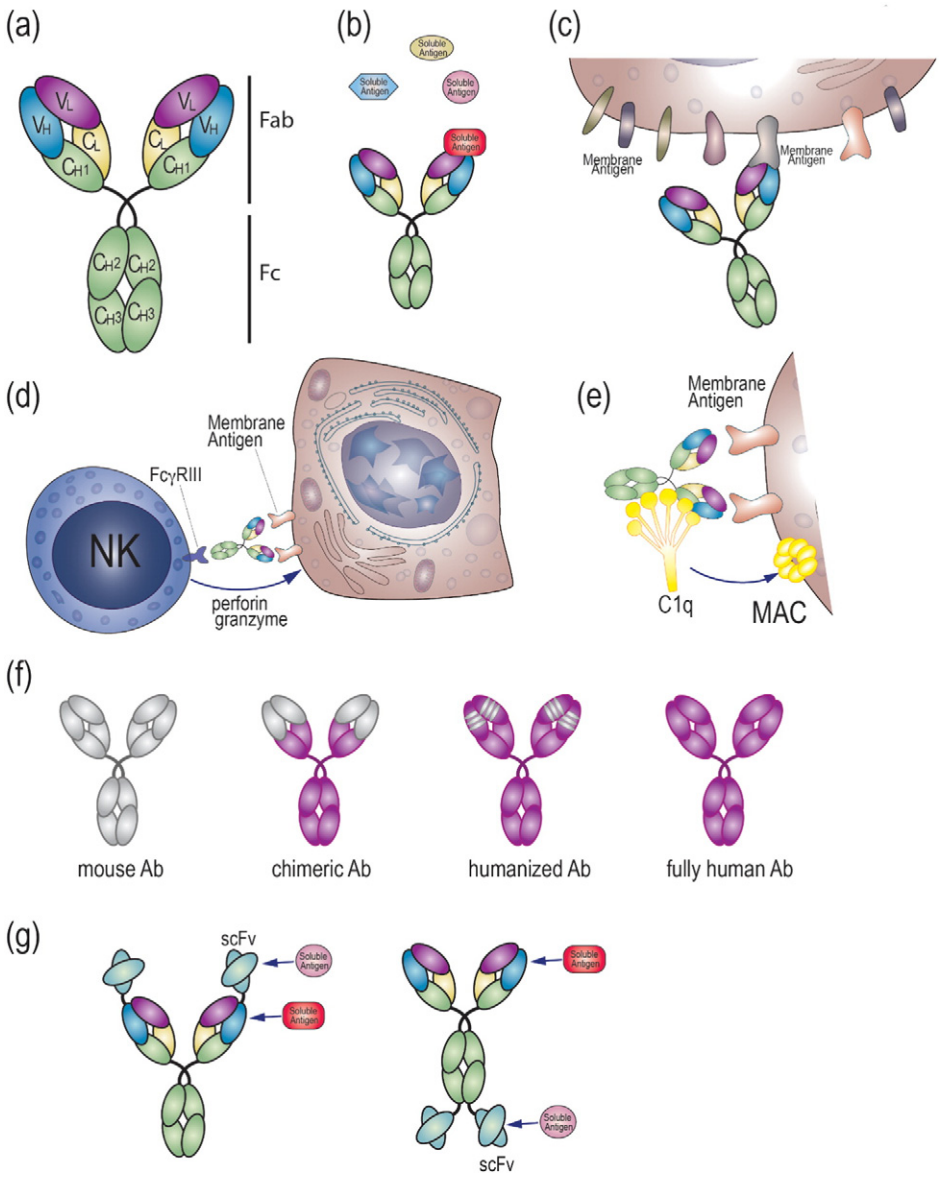


Fig. 2. (a) Monoclonal antibody domain structure. A monoclonal antibody molecule consists of two heavy (H) chains and two light (L) chains that together form an Fab region and an Fc region. The Fab region of the antibody forms a site (paratope) that binds a site (epitope) on a specific antigen. Properties of the Fc region determine whether the antibody will engage the effector functions of neighboring cells and also its half-life. (b–e) Therapeutic antibodies have different modes of action. A monoclonal antibody can specifically bind to and neutralize a soluble (b) or membrane-associated antigen (c). For this function, it is usually desirable to minimize the effector properties of the Fc region. A monoclonal antibody can also bind an antigen specifically expressed on a pathological target-cell population and engage effector cells (e.g., NK cells) via binding of its Fc region to Fcγ receptors (e.g., FcγRIIIa) on the effector cell (d). This triggers production of enzymes from the effector cell that cause death of the target cell. Therapeutically, this mechanism can be utilized to deplete selected pathogenic cell populations. An alternative mechanism to induce target cell death is complement-mediated cytotoxicity (CDC), which engages complement C1q through the "classical pathway", leading to the formation of the membrane-attack complex (MAC) (e). (f) Mouse, chimeric, humanized, and human monoclonal antibodies. The first therapeutic monoclonal antibodies were generated from mouse cells. Chimeric antibodies have a mixture of mouse (gray) and human (mauve) sequences. In humans, mouse and chimeric antibodies can prime the body's immune system to destroy them, resulting in reduced efficacy on repeated treatment. In humanized antibodies, the only murine sequences retained are those directly involved in antigen recognition; but many monoclonal antibodies now entering clinical trials are fully human. Humanized and human monoclonal antibodies are associated with far fewer immunogenic responses. (g) Bispecific formats. Two plausible formats are provided for a bispecific antibody that specifically binds two unrelated antigens. In both formats, one antigen (red) binds via the main antibody framework. Binding of the second antigen (pink) occurs via a single-chain variable fragment (scFv) linked to the main antibody framework at variable locations. Abbreviations: C1q (complement component 1, subcomponent q), Fab (fragment antigen-binding), Fc (fragment crystallizable), FcγRIIIa (fragment crystallizable gamma receptor IIIa), MAC (membrane-attack complex), NK (natural killer cell), TNF (tumor necrosis factor), HER2 (human epidermal growth factor receptor 2), scFv (single-chain variable fragment).

6. Developmental therapeutic antibodies that target eosinophils

6.1. Anti-IL-5 and anti-IL-5Rα

From the choices available, the IL-5 and IL-5Rα pathways appear an obvious approach for selectively targeting eosinophilic inflammation in patients with asthma and COPD. IL-5 is a homodimeric cytokine secreted predominantly by T cells, mast cells, eosinophils, basophils, and the recently described Type 2 innate lymphoid cells (ILC2). The IL-5R comprises an IL-5Rα chain, selectively expressed on eosinophils and basophils, and a common β_c-chain, which it shares with the receptors for IL-3 and GM–CSF (Geijsen et al., 2001; Murphy & Young, 2006). IL-5 binds IL-5Rα subunit with low affinity, but dimerization of IL-5Rα and β_c-subunits generates a high-affinity receptor complex required to transduce intracellular signals upon IL-5 binding (Tavernier et al., 1991; Milburn et al., 1993; Dickason & Huston, 1996; Dickason et al., 1996; Rossjohn et al., 2000). IL-5 is the only cytokine that binds IL-5Rα and activates the high-affinity IL-5R.

Table 1
Summarizing the effects of monoclonal antibody treatments on eosinophils.

Monoclonal antibody	MOA	IgG format affinity for target (K_d)	Effect on eosinophils	Clinical effects
Omalizumab (Xolair®)	Anti-IgE	Humanized IgG_1 K_d = 2.7 nM (Meno-Tetang & Lowe, 2005; Arm et al., 2014) K_d = 7.7 nM (Arm et al., 2014)	Sputum: • Reduction post allergen challenge but not statistically significant compared with placebo (Fahy et al., 1997) • Reduction in patients with mild to moderate allergic asthma (Djukanović et al., 2004) • Nonsignificant reduction compared with placebo in patients with severe allergic asthma (Takaku et al., 2013) Lung tissue: • Statistically significant reduction in lung tissue eosinophils in patients with mild to moderate asthma (Djukanović et al., 2004) and in patients with severe allergic asthma (Riccio et al., 2012) Blood: • Reduction in blood eosinophils post allergen challenge (Fahy et al., 1997) and in patients with moderate to severe allergic asthma (Massanari et al., 2010)	Statistically significant reduction in the number of asthma exacerbations, improvements in lung function and symptoms for patients with moderate to severe allergic asthma (Busse et al., 2001; Solèr et al., 2001)
Mepolizumab (Nucala®)	Anti-IL-5	Humanized IgG_1 K_d = 100 pM (Nucala package insert)	Sputum: • Reduction post allergen challenge in patients with mild asthma (Leckie et al., 2000) • Reduction in patients with severe asthma with eosinophilic inflammation (Haldar et al., 2009; Nair et al., 2009; Pavord et al., 2012) BAL: • Reduction (Flood-Page et al., 2003b) Lung tissue: • Reduction in tissue eosinophils in patients with mild asthma (Flood-Page et al., 2003b) and patients with severe asthma with an eosinophilic inflammation (Haldar et al., 2009) Blood: • Reduction in patients with mild allergic asthma (Leckie et al., 2000; Flood-Page et al., 2003b) and in patients with severe asthma with eosinophilic inflammation (Haldar et al., 2009; Nair et al., 2009; Pavord et al., 2012; Ortega et al., 2014) Bone marrow: • Reduction (Flood-Page et al., 2003b)	Statistically significant reduction in exacerbations, improvement in FEV_1, ACQ-5, and SGRQ for patients with severe, uncontrolled asthma with eosinophilic inflammation (Ortega et al., 2014)
Reslizumab (Cinqair®)	Anti-IL-5	Humanized IgG_4 K_d = 20 pM (Egan et al., 1999; Wechsler et al., 2012) K_d = 81 pM (Walsh, 2013)	Sputum: • Reduction in patients with moderate to severe asthma (Castro et al., 2011) Blood: • Reduction in blood eosinophils (Kips et al., 2003; Castro et al., 2015)	Statistically significant reductions in exacerbations and improvements in lung function, ACQ and AQLQ for patients with severe asthma with eosinophilic inflammation by blood eosinophils ≥ 400/μL (Castro et al., 2015)
Benralizumab	Anti-IL-5Rα with ADCC	Humanized IgG_1 K_d = 11 pM (Kolbeck et al., 2010)	Sputum: • Reduction in patients with mild to moderate asthma (Laviolette et al., 2013) Lung tissue: • Reduction in patients with mild to moderate asthma (Laviolette et al., 2013) Blood: • Reduction in patients with mild (Busse et al., 2010), mild to moderate (Laviolette et al., 2013) and moderate to severe asthma (Castro et al., 2014) Bone marrow: • Reduction in patients with mild to moderate asthma (Laviolette et al., 2013)	Statistically significant reductions in exacerbations and improvements in lung function, asthma symptoms, ACQ-6 and AQLQ in patients with moderate to severe, uncontrolled asthma with eosinophilic inflammation by blood eosinophils ≥ 300/μL (Castro et al., 2014; Bleecker et al., 2016; FitzGerald et al., 2016)
Lebrikizumab	Anti-IL-13	Humanized IgG_4 K_d < 10 pM (Ultsch et al., 2013)	Blood: • Increase in patients with moderate to severe uncontrolled asthma (Corren et al., 2011; Hanania et al., 2015)	Statistically significant reduction in exacerbations and improvement in lung function for patients with moderate to severe asthma and greater concentrations of serum periostin. High blood eosinophils at baseline (≥240/μL) were also predictive of efficacy (Hanania et al., 2015)
Tralokinumab	Anti-IL-13	Fully human IgG_4	Sputum:	Statistically significant reduction in exacerbations and

(continued on next page)

Table 1 (*continued*)

Monoclonal antibody	MOA	IgG format affinity for target (K_d)	Effect on eosinophils	Clinical effects
		K_d = 165 pM (May & Fung., 2015; May et al., 2012; Monk et al., 2005)	• No detectable effect in patients with moderate to severe asthma (Piper et al., 2013) Blood: • Increase in blood eosinophils in patients with moderate to severe asthma (Piper et al., 2013; Brightling et al., 2015a)	improvements in lung function, ACQ-6 and AQLQ for patients with severe asthma with high serum periostin or dipeptidyl peptidase 4 (Brightling et al., 2015)
Dupilumab	Anti-IL-4Rα	Fully human IgG_4 K_d = 68–94 pM (Martin et al., 2009)	Blood: • Overall increase in blood eosinophils in patients with moderate to severe asthma which did not reach statistical significance (Wenzel et al., 2013)	Reduction in exacerbations, improvement in lung function, ACQ-5 and asthma symptom score for patients with moderate to severe asthma with eosinophilic inflammation while tapering ICS therapy (Wenzel et al., 2013)
AMG157/MEDI-9929	Anti-TSLP	Fully human IgG_2 K_d = 15.8–29.2 pM (Gauvreau et al., 2014)	Sputum: • Reduction post allergen challenge in patients with mild asthma (Gauvreau et al., 2014) Blood: • Reduction pre and post allergen challenge in patients with mild asthma (Gauvreau et al., 2014)	Currently in Phase II evaluating the efficacy in patients with moderate to severe asthma
Bertilimumab	Anti-eotaxin-1 (CCL11)	Fully human IgG_4 K_d = 80 pM (Main et al., 2006)	No clinical data to date	No clinical data to date

Abbreviations: ACQ-5, Asthma Control Questionnaire-5; ACQ-6, Asthma Control Questionnaire-6; ADCC, antibody-dependent cell-mediated cytotoxicity; AQLQ, Asthma Quality of Life Questionnaire; FEV_1, forced expiratory volume in 1 s; ICS, inhaled corticosteroids; IgE, immunoglobulin E; IgG_1, immunoglobulin G_1; IgG_2, immunoglobulin G_2; IgG_4, immunoglobulin G_4; IL-5, interleukin-5; IL-5Rα, interleukin-5 receptor α; MOA, mechanism of action; SGRQ, St. George's Respiratory Questionnaire.

IL-5 has long been recognized as the cytokine most specific to the eosinophil lineage; it is strongly involved in all stages of eosinophil development, as well as many aspects of eosinophil pathobiology (Sanderson, 1992; Rådinger & Lötvall, 2009), making it an attractive target for an anti-eosinophil biologic. It plays a central part in differentiation and maturation of eosinophils in bone marrow, and IL-5 priming induces eosinophil locomotor activity, suggesting that it might play a role in eosinophil recruitment to inflamed pulmonary tissues (Sehmi et al., 1992; Wen et al., 2013; Larose et al., 2014). IL-5 stimulates activation and degranulation of eosinophils; enhances their survival in tissues (Ochiai et al., 1997; Huang et al., 2005); and promotes eosinophilic precursors to proliferate, differentiate and function independently in various tissues, including the lung (Robinson et al., 1999a, 1999b; Lu et al., 2010; Dorman et al., 2004a, 2004b; Rådinger & Lötvall, 2009; Fulkerson et al., 2014). IL-5 expression is elevated in bronchoalveolar lavage fluid and bronchial biopsies from asthmatic patients with eosinophilic pulmonary inflammation (Hamid et al., 1991; Kim et al., 2003; Huang et al., 2005). Inhalation of IL-5 by patients with asthma causes sputum eosinophilia and airway hyper-responsiveness (Shi et al., 1998).

Despite the clear association of IL-5 and eosinophils with asthma, developing IL-5-directed therapeutics has proven much more difficult than originally anticipated. In particular, the early clinical trial results with IL-5-directed mAbs in "all-comers" asthma trials proved to be extremely disappointing (Leckie et al., 2000; Flood-Page et al., 2003a, 2007; Kips et al., 2003). Nevertheless, careful subpopulation responder analysis has led to the identification that severe asthma patients with evidence of eosinophilic inflammation in the airway have marked reduction in eosinophilic inflammation and clinical benefits when treated with an anti-IL-5 monoclonal antibody (Haldar et al., 2009; Nair et al., 2009). Therefore, IL-5 is an important cytokine contributing to eosinophilic inflammation in these patients.

Mepolizumab (Nucala®; SB-240563; GSK) is a humanized mAb of the immunoglobulin IgG_1/kappa isotype with dosage-proportional pharmacokinetics and a terminal half-life of approximately 21 days in patients with asthma. Maximum plasma concentrations occurred 0.5–4.8 h following infusion, and mepolizumab was well-absorbed (Smith et al., 2011). An early allergen challenge study of patients with mild asthma who received mepolizumab demonstrated pronounced long-term suppression of circulating eosinophils and significantly lowered sputum eosinophils, but had no effect on the allergen-induced early or late asthmatic response or histamine-induced AHR (Leckie et al., 2000). A separate study conducted in patients with mild, controlled, atopic asthma evaluated the effect of mepolizumab on eosinophil numbers and eosinophil activation products in the lung tissue. In this study, 3 months of treatment with mepolizumab induced substantial reductions of eosinophils in blood, bronchoalveolar lavage fluid, and bone marrow. However, the median reduction in mucosal airway eosinophils was only 55%, and eosinophil activation products in the lung tissue were similar in patients treated with mepolizumab compared with placebo. Interestingly, the investigators reported no significant effect of mepolizumab on number of basophils, the only other cell-type in human expressing the IL-5R and responding to IL-5 (Denburg et al., 1991), in the lung tissue. In addition, 3 months of treatment with mepolizumab had no statistically significant effect compared with placebo on any of the clinical endpoints measured (Flood-Page et al., 2003a). Treatment with mepolizumab resulted in a statistically significant reduction in extracellular matrix protein deposition compared with placebo, suggesting eosinophils may contribute to airway tissue remodeling (Flood-Page et al., 2003b). In a larger study conducted in patients with asthma and persistent symptoms despite ICS therapy, mepolizumab treatment did not significantly improve clinical endpoints (e.g., FEV_1, β-agonist use, symptom score, exacerbation rates), although there was a not statistically significant numerical decrease in exacerbation rates in the group receiving the greatest mepolizumab dosage. Thus, mepolizumab treatment did not appear to add significant clinical benefit in patients with asthma whose symptoms persisted despite corticosteroid therapy, although it suggested the need for further studies on exacerbation rates (Flood-Page et al., 2007). However, these early clinical trials did not preselect patients with eosinophilic airway inflammation, and they largely focused on clinical outcomes not closely associated with eosinophilic airway inflammation.

A major breakthrough came with two small proof-of-concept studies demonstrating that treatment with mepolizumab reduced the risk of exacerbations in patients with severe, refractory eosinophilic asthma and a history of severe exacerbations. In these studies,

eosinophilic asthma was defined by a sputum eosinophil count of at least 3%. In the first study, mepolizumab treatment resulted in a 2-fold reduction in lung tissue eosinophils and a significant 43% (95% CI 8–68%) reduction in the annual exacerbation rate compared with placebo, but had no significant effects on symptoms, FEV_1, or AHR (Haldar et al., 2009). This key finding was confirmed in the second smaller study (Nair et al., 2009) conducted in prednisone-dependent patients with asthma and sputum eosinophilia. Ten of 11 patients who had received placebo experienced asthma exacerbations, compared with 1 of 9 patients who received 5 monthly infusions of mepolizumab (750 mg). Patients receiving mepolizumab were able to reduce their daily dosages of prednisone by a mean of 83.8% of their maximum possible dosages, while maintaining efficacy. The much larger 621-patient DREAM clinical trial corroborated these initial findings with mepolizumab in patients with severe, uncontrolled asthma and evidence of eosinophilic inflammation (peripheral blood eosinophil count of at least 300/μL; Pavord et al., 2012). The annual number of clinically significant exacerbations per patient was reduced by 48% (95% CI 31–61%) in patients who had received mepolizumab 75 mg intravenously every 4 weeks compared with placebo, accompanied by a reduction in blood eosinophil counts (geometric mean ratio compared with placebo (0.22 [0.18–0.27]). The utility of measuring blood eosinophil counts is of particular note, for early work had suggested that it might be necessary to determine eosinophil counts in sputum, lavage, or lung biopsy, all of which are impractical in usual clinical practice outside of specialist research centers.

All tested mepolizumab dosages (75 mg, 250 mg, and 750 mg intravenously) significantly reduced the number of exacerbations and time to first exacerbation, whereas the effects of mepolizumab on secondary endpoints of FEV_1 and ACQ (Asthma Control Questionnaire) scores were generally similar to placebo. The differential effects of mepolizumab on exacerbations, lung function, and day-to-day clinical manifestations of asthma (FEV_1, ACQ scores) prompted a discussion of the extent to which these manifestations are mechanistically distinct features of severe asthma patient populations. Although the DREAM study did not demonstrate improvement in FEV_1 or ACQ scores, the Mepolizumab as Adjunctive Therapy in Patients with Severe Asthma (MENSA) Phase III trial (Ortega et al., 2014) demonstrated improvement in both of these parameters. Compared with placebo, 100-mg subcutaneous mepolizumab demonstrated improvements in FEV_1 of 98 mL (95% CI 11–184) and ACQ-5 score of −0.44 (−0.63 to −0.25), as well as a relative reduction in annual exacerbation rate of 53% (36–65). In this Phase III trial, eosinophilic inflammation was defined as a peripheral blood eosinophil count of at least 150/μL at screening or a historic eosinophil count of at least 300/μL in the previous 12 months. A *post-hoc* analysis of data from the DREAM and MENSA studies reported a relationship between baseline blood eosinophil count and clinical efficacy of mepolizumab (Ortega et al., 2016). Reductions in exacerbation rates with mepolizumab increased with baseline eosinophil count, from 52% (95% CI 42–61) for patients with a count of at least 150/μL to 70% (60–77) for patients with a count of at least 500/μL.

A recent review of eight mepolizumab randomized clinical trials involving 1707 participants, which included the MENSA study, concluded that patients with severe asthma with eosinophilic inflammation receiving mepolizumab had reduced exacerbations and improved health-related quality of life (Powell et al., 2015). However, this review concluded that there was no significant benefit on lung function, asthma symptom scores, cough scores, or AHR.

Despite its demonstrable neutralization of IL-5, mepolizumab does not completely deplete eosinophils in the airway mucosa in patients with either mild to moderate or severe asthma (Flood-Page et al., 2003a; Haldar et al., 2009). This may be explained by the fact that, in humans, eosinophil survival factors other than IL-5, such as GM–CSF are involved in tissue survival (Simon & Blaser, 1995; Yousefi et al., 1996; Simon et al., 1997; Shen & Malter, 2015). GM–CSF enhances eosinophil survival in allergic nasal polyps. GM–CSF mRNA, not IL-5 mRNA, is increased in individuals with non-allergic chronic sinusitis and nasal polyps, and tissue eosinophilia has a stronger correlation with GM–CSF than with IL-5 expression. In patients with allergic chronic sinusitis and nasal polyps, eosinophilia had a stronger correlation with GM–CSF and IL-3 expression than with IL-5 expression (Hamilos et al., 1993; Hamilos et al., 1995). Thus, strategies that deplete eosinophils more profoundly than anti-IL-5 treatments may prove to be even more effective.

The Food and Drug Administration and the European Medicines Agency approved mepolizumab in late 2015. In the United States, mepolizumab is indicated for the add-on maintenance treatment of patients with severe asthma aged 12 years and older and with an eosinophilic phenotype, and, in Europe, is indicated as an add-on treatment for severe refractory eosinophilic asthma in adult patients (Nucala European Union Summary of Product Characteristics, 2015; Nucala Package Insert, 2015). Mepolizumab is currently being studied in three Phase III clinical trials in patients with COPD with eosinophilic airway inflammation (NCT01463644, NCT02105948, NCT02105961).

Reslizumab (Cinquair®; SCH55700; Teva) is a humanized rat anti-IL-5 mAb of the immunoglobulin IgG_4/kappa subtype. The terminal half-life for reslizumab is 24.5–30.1 days, with the maximal concentration obtained 6.9 h after dosing (Kips et al., 2003). The clinical development path of reslizumab started on a similarly disappointing note as that of mepolizumab. In an early clinical study, treatment with a single dose of reslizumab reduced circulating eosinophils but provided no overall significant improvement in lung function (FEV_1) and other clinical indices of disease activity in patients with severe persistent asthma (Kips et al., 2003). However, in a subsequent clinical trial that recognized the importance of selecting patients with uncontrolled asthma with eosinophilic inflammation, reslizumab 3.0 mg/kg administered every 4 weeks for 12 weeks induced a significant improvement in FEV_1 of 0.24 L (95% CI 0.09–0.39) compared with placebo and a non-statistically significant reduction in exacerbations in these patients (Castro et al., 2011). In two subsequent multicenter Phase III studies, reslizumab 3.0 mg/kg administered every 4 weeks for 48 weeks demonstrated improvements compared with placebo for exacerbations (rate reduction 54% [95% CI 42–63%]), lung function (0.11 L [0.067–0.15]), ACQ-7 (−0.25 [−0.34 to 0.16]), and health-related quality of life scores (0.23 [0.16–0.39]) (Castro et al., 2015). Although the improvement in asthma control and lung function measures were more striking than those in the mepolizumab DREAM or MENSA studies, this reslizumab study had a peripheral blood eosinophil count cutoff of at least 400/μL eosinophils, compared with 150/μL in the mepolizumab studies. Another study recently found a positive relationship between baseline blood eosinophil counts and FEV_1 improvement at Week 16 with reslizumab treatment (slope: 0.0229) for patients with poorly controlled asthma (Corren et al., 2016).

Efficacy differences observed in clinical trials between mepolizumab and reslizumab are probably not a result of their pharmacologic properties, which are similar. Both have a high affinity to IL-5, with a dissociation constant of 100 pM for mepolizumab and 81 pM for reslizumab (Egan et al., 1995; Hart et al., 2001; Nucala Package Insert, 2015). Both antibodies have low picomolar activity in inhibiting IL-5 activity in cell models (Egan et al., 1995; Hart et al., 2001; Nucala Package Insert, 2015).

The Food and Drug Administration and the European Medicines Agency approved reslizumab in March 2016. In the United States, reslizumab is indicated for add-on maintenance treatment of patients with severe asthma aged 18 years and older, and with an eosinophilic phenotype (Cinqair Package Insert, 2016). Reslizumab received a positive opinion recommending marketing authorization from the European Medicines Agency's (EMA) Committee for Medicinal Products for Human Use (CHMP) in June 2016, and has been submitted to and is currently under review by Health Canada.

Benralizumab (AstraZeneca, MedImmune) is a humanized, afucosylated monoclonal antibody (IgG_1 k) that binds with high affinity to IL-5Rα and rapidly depletes eosinophils and basophils through ADCC.

Benralizumab lacks a fucose sugar residue in the CH2 region of its Fc domain, enhancing its ability to bind the main activating human Fcγ-receptor IIIa (CD16) on natural killer (NK) cells (Kolbeck et al., 2010), the importance of which has been demonstrated in ADCC assays using primary human eosinophils and NK cells. In contrast with strategies such as *anti*-FAS (CD95) antibodies, which provoke eosinophils to release free eosinophilic granules (FEGs) that damage tissue (Uller et al., 2005), eosinophil apoptosis induced by benralizumab is not associated with eosinophil degranulation in vitro (Kolbeck et al., 2010). Benralizumab has a mean elimination half-life of approximately 2 to 3 weeks (Busse et al., 2010). Its mean volume of distribution is greater than the plasma volume, potentially indicating binding to IL-5Rα cells and/or moderate extravascular tissue penetration (Busse et al., 2010).

In a Phase I study in patients with asthma, benralizumab given as single IV doses produced a rapid and nearly complete depletion of peripheral blood eosinophils persisting for at least 84 days, with corresponding reductions in serum eosinophil activation products ECP and EDN (Busse et al., 2010; Pham et al., 2016). A Phase II, multiple-ascending-dosage safety study in adult patients with asthma using a subcutaneous formulation of benralizumab confirmed the pharmacokinetic and pharmacodynamic properties observed with the IV dosing study (Gossage et al., 2010). In agreement with in-vitro studies, benralizumab induced reversible eosinopenia in patients with asthma without inducing eosinophil degranulation. For patients with mild to moderate asthma and ≥2.5% sputum eosinophilia despite ICS therapy, treatment with benralizumab subcutaneously for 3 months demonstrated substantial depletion of eosinophils in blood, sputum, bone marrow, and lung tissue (combined 100-mg and 200-mg dosage groups; Laviolette et al., 2013). In a Phase IIb study of patients with uncontrolled asthma, benralizumab, given subcutaneously at dosages up to 100 mg every 8 weeks for 1 year had an acceptable safety profile, with few serious treatment-related adverse events. Benralizumab 100 mg every 8 weeks reduced the acute exacerbation rate by 41% (95% CI 11–60) compared with placebo for patients with baseline blood eosinophils ≥ 300 cells/μL, but patients without eosinophilia did not exhibit this same benefit. In the cohorts with eosinophilia, all dosages of benralizumab resulted in improvements in the secondary endpoints FEV_1 and ACQ-6, compared with placebo (Castro et al., 2014). In a subsequent study, a single dose of benralizumab administered in the outpatient setting 7 days after patients presented to the emergency department with an asthma exacerbation reduced the subsequent asthma exacerbation rate by 49% and exacerbations requiring hospitalization by 60% compared with placebo (Nowak et al., 2015).

Benralizumab achieved the primary endpoints in two pivotal Phase III registrational trials (SIROCCO and CALIMA), demonstrating significant reductions in annual asthma exacerbation rates and improved lung function compared with placebo, and was well-tolerated (Bleecker et al., 2016; FitzGerald et al., 2016).

A small Phase IIa study evaluated benralizumab in patients with moderate to very severe COPD, a history of previous exacerbations, and sputum eosinophilia. Following subcutaneous administration of benralizumab 100 mg every 8 weeks (after three doses 4 weeks apart), blood and sputum eosinophils were profoundly depleted from the first measurement time point through 1 year of treatment, achieving much greater eosinophil suppression than in earlier studies of inhaled or oral corticosteroids (Brightling et al., 2014). Benralizumab did not meet its primary endpoint, a reduction in exacerbation rate at Week 56 in the overall population. A prespecified subanalysis indicated that patients with elevated blood eosinophils (≥200 cells/μL) treated with benralizumab had a nonsignificant 31% reduction in COPD exacerbation rate compared with placebo. Patients with elevated blood eosinophils also exhibited significant improvement in FEV_1 (0.29 L; 95% CI 0.02–0.55), whereas patients without elevated eosinophils did not. These findings supported further clinical development of benralizumab to investigate potentially beneficial clinical effects in a subgroup of patients with COPD. Two Phase III studies are currently underway in patients with COPD (NCT02138916, NCT02155660).

6.2. Anti-IL-5/anti-IL-5R antibodies: Are they all the same?

The distinctive mechanisms of actions of benralizumab, which depletes eosinophils and basophils through enhanced ADCC, and the IL-5 ligand blocking antibodies mepolizumab and reslizumab may produce differential ablation of eosinophils in key tissues.

Benralizumab, reslizumab, and mepolizumab all suppress blood eosinophil counts in patients with asthma. In sputum, benralizumab and mepolizumab reduce eosinophils to similar degrees. Benralizumab (100 mg or 200 mg, subcutaneous once monthly) demonstrated a combined 89.9% decrease in sputum eosinophils at Day 28 (Laviolette et al., 2013). In the DREAM study, the top dosage of mepolizumab (750 mg IV once monthly) had a similar high degree of suppression (88%) at 52 weeks, but with a wide variability (Pavord et al., 2012). Speed of eosinophil depletion differs in benralizumab vs. mepolizumab. Benralizumab depletes peripheral blood eosinophils rapidly, with a peak reduction within 24 h (Laviolette et al., 2013). Mepolizumab induces a gradual reduction that peaks at 4 weeks after dosing (Flood-Page et al., 2003a). In addition, the ability of these agents to suppress bone marrow eosinophils and eosinophil precursors differs. For four patients with asthma receiving benralizumab, eosinophils and eosinophil precursors were undetectable in all bone marrow aspirates (Laviolette et al., 2013). In contrast, three intravenous mepolizumab dosages resulted in a 52% median reduction in bone marrow eosinophil count (Flood-Page et al., 2003a). Differences in study design, study populations, and eosinophil inclusion criteria between the studies may have contributed to the different results.

Airway tissue eosinophils are the key effector cells in causing airway damage, and here significant differences emerge between benralizumab and mepolizumab. Three monthly infusions of 750 mg of intravenous mepolizumab resulted in a median decrease in airway mucosal eosinophil counts of only 55% (Flood-Page et al., 2003a). In a subsequent study, 12 monthly intravenous infusions of 750 mg of mepolizumab in patients with severe refractory asthma with sputum eosinophilia also produced a similar magnitude of reduction in lung tissue eosinophils (Haldar et al., 2009). In contrast, three subcutaneous benralizumab dosages (100 mg or 200 mg once monthly) demonstrated a median reduction of 95.8% in mucosal airway eosinophil counts (Laviolette et al., 2013). However, the sample size in this study was very small, and this finding requires further exploration. The decreased dependence of airway tissue eosinophils on IL-5 for survival may explain their incomplete removal by the anti-IL-5 therapy mepolizumab. Activated eosinophils in the airways of patients with asthma exhibit decreased IL-5Rα surface expression (Liu et al., 2002a,b; Gregory et al., 2003). Furthermore, cytokines such as IL-3 and GM–CSF persist in the airways and can aid tissue eosinophil survival. In animal models of allergy, more effective eosinophil depletion can be achieved by combining IL-5 inhibition with blockade of GM–CSF and IL-3 or the eotaxin receptor CCR3 (Nishinakamura et al., 1996; Foster et al., 2001). In contrast, benralizumab would be expected to cause tissue eosinophil apoptosis regardless of the presence of eosinophil survival factors. Furthermore, benralizumab can drive eosinophil apoptosis at low IL-5R densities because the ADCC mechanism by which it works is relatively insensitive to surface density of target receptors (van Meerten et al., 2006). Immunohistochemistry studies of lung biopsies from patients with asthma indicate that benralizumab stains more than 90% of eosinophils, suggesting that IL-5R receptor density is sufficient for benralizumab engagement in this key location (Kolbeck et al., 2010). The pronounced depletion of mucosal airway eosinophils with benralizumab is likely a result of ADCC decreasing the number of eosinophils available for trafficking into the lung and directly reducing eosinophil count in the lung. However, further kinetic studies in patients, with accounting for lung tissue $T_{½}$ of eosinophils, would be required to establish the main

mechanism. In summary, activated airway tissue eosinophils may be less dependent on IL-5 for survival, but they are still susceptible to the effects of a highly potent, eosinophil-depleting, anti-IL-5R mAb.

Benralizumab also depletes other potentially pathogenic cell populations expressing IL-5R, such as basophils, as suggested by in-vitro studies (Kolbeck et al., 2010). In a small double-blind, placebo-controlled trial of patients with asthma, median estimates of basophil count were reduced by 74% at Day 84, following three monthly subcutaneous doses of benralizumab (Laviolette et al., 2013). Basophils have been repeatedly implicated in allergic airway disease (Gauvreau et al., 2000; MacGlashan et al., 2002; Salter et al., 2015), but it is not known whether the depletion of basophils contributes to the therapeutic benefit of benralizumab.

The apparent pharmacodynamic differences in the magnitude of eosinophil depletion in bone marrow and lung tissue and the additional effect of benralizumab to deplete basophils are interesting. It is unknown whether these will translate into differences in the clinical efficacy of these different mechanisms of action for patients with severe, uncontrolled asthma with eosinophilic inflammation, patients with COPD and eosinophilic inflammation, or patients with other diseases characterized by eosinophilic inflammation.

Finally, subcutaneous administrations of benralizumab and mepolizumab may provide patients with convenience in comparison with reslizumab, which is currently administered intravenously.

6.3. Anti-IgE monoclonal antibodies

Allergens are key triggers of asthma and, with allergic asthma, a common phenotype in patients with asthma (Global Initiative for Asthma, 2016). Allergens, recognized by dendritic cells, lead to activation of Th2 cells, B-cell proliferation, and IgE production. IgE binds to the high-affinity IgE receptor, FcεRI, expressed on basophils and mast cells. Cross-linking of IgE, upon subsequent exposure to specific allergens results in activation and degranulation of basophils and mast cells and release of pro-inflammatory cytokines, chemokines, prostanoids, and peptidyl leukotrienes that can propagate eosinophilic inflammation. Therefore, blocking IgE to prevent this reaction is a potential mechanism for preventing eosinophilic inflammation.

Omalizumab (Xolair®; Roche Genentech and Novartis) is a humanized IgG_1 *anti*-IgE mAb that inhibits binding of IgE to its low- and high-affinity receptors, CD23 and FcεRI, respectively. In patients with asthma, it decreases free IgE concentrations and downregulates expression of high affinity IgE receptors. In 2003, omalizumab became the first Food and Drug Administration–approved biologic for asthma treatment when it was approved for the treatment of patients with moderate to severe persistent allergic asthma whose disease is not adequately controlled by ICS alone. The dosage of omalizumab is complex and determined by a patient's baseline IgE (IU/ml) and body weight (kg). Furthermore, the criteria for dosing, based on baseline IgE concentrations, differ in approved territories (Xolair [European Union Summary of Product Characteristics], n.d.; Xolair, United States package insert, 2014). Omalizumab decreases the rate of asthma exacerbations, annualized rates of hospital admissions, total emergency department visits, rescue therapy use, and ICS dosage.

Several studies have demonstrated that omalizumab reduces numbers of peripheral, sputum and bronchial submucosal eosinophils in patients with asthma and allergies (Fahy et al., 1997; Djukanović et al., 2004; Massanari et al., 2010; Riccio et al., 2012; Takaku et al., 2013). Skiepko et al. demonstrated that patients with asthma and allergies who exhibited a strong reduction in blood eosinophil counts in response to omalizumab had a lower exacerbation rate in the subsequent 12 months on treatment (Skiepko et al., 2014). In a much larger study of patients with uncontrolled asthma with normal lung function treated with omalizumab, a baseline peripheral eosinophil count ≥ 300/μL was associated with a reduction in asthma exacerbations versus placebo. On the other hand, patients with low baseline eosinophil counts exhibited no improvement in exacerbations (Busse et al., 2013). Another large study in omalizumab-treated patients with severe, persistent asthma and allergies reached a similar conclusion (Hanania et al., 2013). In these studies, patient IgE concentrations were 30–700 IU/mL (Hanania et al., 2013; Skiepko et al., 2014) or 30–1300 IU/mL (Busse et al., 2013). Collectively, these studies suggest that omalizumab can suppress eosinophil counts in some patients with asthma and that patients with greater baseline eosinophils receive greater benefit from omalizumab therapy. The mechanism by which anti-IgE suppresses eosinophil recruitment and the extent of airway eosinophil reduction are areas for further investigation that may inform the potential overlap between anti-IgE and anti-IL-5 therapies. Reduction in exacerbations with anti-IL-5 therapy did not correlate with atopic status or IgE concentrations (Pavord et al., 2012), a finding that provides a basis for differentiation between anti-IL-5 and anti-IgE therapies. Nevertheless, a recent study of the National Health and Nutrition Examination Survey (2005–2006) indicated a significant overlap of eosinophilic and atopic phenotypes in patients with severe asthma (Tran et al., 2016). Therefore, further research will be required to understand which patients may gain optimal treatment benefit from anti-IgE therapy versus anti-eosinophilic treatment with anti-IL-5 monoclonal antibodies or benralizumab.

New-generation anti-IgE monoclonal antibodies now in clinical development may demonstrate more potent effects on IgE concentrations. One such monoclonal antibody is QGE031 (ligelizumab), which is currently in Phase II clinical trials for asthma (NCT02336425).

6.4. Anti-IL-13 and anti-IL-4Rα monoclonal antibodies

IL-13 is a Th2 cytokine that enhances mucus production, goblet cell hyperplasia, smooth muscle cell contraction, airway remodeling, and AHR (Rael & Lockley, 2011; Corren, 2013). In conjunction with IL-4, IL-13 generates IgE class switching. Therapeutic antibodies directed against IL-13 specifically interfere with the activity of IL-13, whereas IL-4Rα inhibition antagonizes the activity of both IL-4 and IL-13 because of the use of this receptor chain by both cytokines. Eosinophils are a source of IL-4 and IL-13 and respond to IL-4 and IL-13 with activation, survival and cytokine expression. IL-4 and IL-13 indirectly regulate eosinophils through their pleiotropic activities on many cell types, which result in the production of soluble mediators and cell surface receptors interacting with eosinophils. For a comprehensive summary of the pathological activities and targeting strategies for both cytokines, we would like to direct the reader to a recent review (May & Fung, 2015).

Tralokinumab (AstraZeneca, MedImmune) is a fully human, IgG_4 anti-IL-13 mAb that has entered Phase III clinical trials in asthma (NCT02161757, NCT02194699, NCT02281357). Tralokinumab was initially evaluated in 194 patients with moderate to severe, uncontrolled asthma at subcutaneous dosages of 150 mg, 300 mg, or 600 mg every other week for 12 weeks. Tralokinumab failed to affect ACQ-6, but it dosage-dependently improved FEV_1 by 150 mL and reduced short-acting β agonist use by 0.5 puffs per day compared with placebo. In a *post-hoc* analysis, patients who were positive for sputum IL-13 achieved better outcomes than patients who were negative (Piper et al., 2013). In a subsequent, larger Phase IIb study, tralokinumab at the greatest dosage evaluated (300 mg subcutaneously every other week for 52 weeks), did not significantly reduce asthma exacerbations in "all-comers" with severe, uncontrolled asthma, but it demonstrated a 67% reduction in exacerbation rate in patients with reversible asthma and high periostin concentrations (Brightling et al., 2015a). At the same dosage, for patients with high periostin concentrations, tralokinumab significantly improved FEV_1 and ACQ-6 versus placebo (Brightling et al., 2015b). Elevated serum DPP-4 has also been proposed as a surrogate biomarker of IL-13 pathway activation in the lung, and an enhanced response to tralokinumab was reported for patients with high baseline DPP-4 concentrations in this Phase IIb study. Interestingly, tralokinumab induced elevated blood eosinophil numbers in the study

and in a previous smaller clinical study of patients with moderate to severe asthma (Piper et al., 2013), but it did not provide an effect on sputum eosinophils (Piper et al., 2013). The Phase II MESOS study (NCT02449473) aims to better understand the mechanism of action of tralokinumab in improving asthma control and investigate the hypothesis that tralokinumab inhibits eosinophil elevation in the lung by interfering with eosinophil trafficking to the lung (Brightling et al., 2015b).

Periostin is a serum biomarker induced by IL-4 and IL-13 (Yuyama et al., 2002; Woodruff et al., 2007) in airway epithelial cells that may successfully predict response of patients with asthma to anti-IL-13 monoclonal antibodies. Hence, in addition to elevated numbers of eosinophils, periostin represents another useful biomarker to tailor treatment for patients with uncontrolled severe asthma.

Lebrikizumab (Roche) is a humanized, IgG_4, anti-IL-13 mAb being studied in Phase III clinical trials in asthma (NCT01867125, NCT01868061, NCT01875003, NCT01987492, NCT02099656, NCT02104674) and a Phase II study in COPD (NCT02546700). Clinical trials that included a small, lung-allergen provocation study of patients with mild asthma also evaluated lebrikizumab. In one study, lebrikizumab non-significantly inhibited the late-phase response by 48% and suppressed serum IgE, CCL13 (MCP-4) and CCL17 (thymus- and activation-regulated chemokine/TARC) concentrations by approximately 25% after 12 weeks of 5 mg/kg subcutaneous administrations (Scheerens et al., 2014). Suppression of these biomarkers, including fractional exhaled nitric oxide (FeNO), by lebrikizumab is a common finding across several clinical studies in patients with mild to poorly controlled, severe asthma (Corren et al., 2011; Noonan et al., 2013; Scheerens et al., 2014; Hanania et al., 2015). In a study of steroid-naïve patients with asthma, lebrikizumab, when dosed subcutaneously at 125 mg, 250 mg or 500 mg every 4 weeks for 12 weeks, protected patients from treatment failure (need for ICS/OCS) and modestly improved FEV_1, but it did not affect ACQ. Interestingly, for this patient population, none of these outcomes was associated with serum periostin status (Noonan et al., 2013). When studied in 219 patients with asthma that was uncontrolled despite medium–high dosages of ICS, 250 mg lebrikizumab (subcutaneous monthly injections) improved FEV_1 by 8.2% and reduced the rate of severe exacerbations by 67% vs. placebo for patients with high serum periostin (Corren et al., 2011). In pooled patient cohorts of two subsequent clinical trials in patients with uncontrolled asthma despite ICS (LUTE and VERSE), lebrikizumab dosages of 37.5 mg, 125 mg, and 250 mg monthly reduced the exacerbation rate in patients with high periostin by 81%, 77%, and 22%, respectively (Hanania et al., 2015). However, of two identical Phase III studies of patients with severe asthma (LAVOLTA I and II), only one study met its primary endpoint of lebrikizumab treatment significantly reducing exacerbations in the primary population of patients with periostin ≥ 50 ng/mL or blood eosinophils ≥ 300 cells/μL (i.e., biomarker-high patients) (Hanania et al., 2016). For these biomarker-high patients, lebrikizumab did not consistently demonstrate reductions in asthma exacerbations.

Dupilumab (Sanofi, Phase III) is a fully human, mAb to the IL-4Rα subunit, and thus blocks both IL-4 and IL-13 signaling. IL-4 is key to polarization and maintenance of Th2 cells and drives Ig class switching from IgM to IgE antibodies in B cells. In a Phase IIa study, patients with asthma on high-dosage ICS/LABA with elevated blood (≥300/μL) or sputum eosinophils (≥3%) were administered weekly subcutaneous dosages of 300 mg of dupilumab for 12 weeks (Wenzel et al., 2013). After Week 4, LABA dosing was discontinued, and after Week 6, ICS therapies were tapered. Dupilumab treatment resulted in a 210-mL improvement in FEV_1 and a 1.04 improvement in ACQ-5 score, in contrast with a 20-mL worsening in FEV_1 and a 0.66 improvement in ACQ-5 score for placebo-treated patients. By Week 12, 6% of the dupilumab cohort and 44% of the placebo cohort had experienced an exacerbation, clearly demonstrating dupilumab's prevention of ICS-withdrawal-induced exacerbations. Similar to the anti-IL-13 monoclonal antibodies, dupilumab also suppressed TARC, eotaxin-3, FeNO and IgE concentrations. The majority of patients treated with dupilumab exhibited little or no change in blood eosinophil counts. However, some patients also exhibited increases in blood eosinophil counts, which resulted in an overall trend for a mean increase in blood eosinophils at the end of the 12-week dupilumab treatment period that did not reach statistical significance (Wenzel et al., 2013a). In a Phase IIb dose-ranging trial in patients with asthma uncontrolled with medium- to high-dosage ICS/LABA were treated for 24 weeks with 200-mg or 300-mg dupilumab every 2 or 4 weeks or placebo (Wenzel et al., 2016). Improvements in lung function and exacerbation rate were greater for the dosages given every 2 weeks compared with the other treatment groups for both the overall population and the subgroups with blood eosinophil counts either ≥300 or <300 cells/μL at Week 12. For the patients who had received dupilumab every 2 weeks, the least-square mean difference versus placebo in FEV_1 change from baseline was 0.16 L–0.20 L. Risk reduction versus placebo for the annualized severe exacerbation rate was 59.9%–67.6% (Wenzel et al., 2016). Dupilumab is currently being evaluated in Phase III studies of patients with asthma (NCT02414854, NCT02528214).

Noteworthy observations from these studies were that patients not on long-term OCS with baseline FEV_1 reversibility ≥12% responded better to anti-IL-13 mAb treatment than the overall population (Brightling et al., 2015b). In addition, treatment with tralokinumab, lebrikizumab, or dupilumab was more efficacious (i.e., improvements in FEV_1) for patients with increased blood eosinophil counts ≥240 to ≥300 cells/μL (Piper et al., 2013; Hanania et al., 2015; Wenzel et al., 2016).

Interestingly, patients receiving anti-IL-13 or anti-IL-4Rα therapies (tralokinumab, lebrikizumab, and dupilumab) exhibited moderately increased blood eosinophil counts (Corren et al., 2011; Piper et al., 2013; Wenzel et al., 2013; Hanania et al., 2015; Brightling et al., 2015a). This finding may reflect inhibition of eosinophil-recruiting chemokines by blocking IL-13 biological activity, reducing migration of eosinophils from the blood to the lungs. Data regarding the effect of anti-IL-13 therapy on tissue, sputum, and lavage eosinophils are eagerly awaited (NCT02099656).

The absence of evidence for profound peripheral eosinophil suppression in patients treated with anti-IL-13 and anti-IL-4Rα therapies points to fundamental differences in their mechanisms of action compared with anti-IL-5 and anti-IL-5Rα therapies. If so, then this observation could point to only a limited overlap with anti-IL-5/anti-IL-5R therapies. The efficacy of anti-IL-5 and anti-IL-5R therapies for asthma patients with high concentrations of biomarkers for IL-13 pathway activation suggests that further exploration may help to differentiate between anti-IL-13 and anti-IL-5 therapies and provide a basis for co-administration of these therapies in specific asthma subpopulations.

6.5. Other therapeutic antibodies

Anti-TSLP (AMG157/ MEDI-9929, Amgen, AstraZeneca, MedImmune) is a fully human *anti*-TSLP mAb inhibiting TSLP activity. TSLP is a cytokine mainly produced from structural cells, including airway epithelial cells, in response to pro-inflammatory stimuli. TSLP induces Th2 inflammation through its activities on many cell types, of which the most prominent are the induction of OX40L/OX40 signaling and the production of Th2 cytokines by dendritic cells, mast cells, ILC2 and $CrTh2^+$ $CD4^+$ T cells. AMG157 attenuated most measures of allergen-induced early and late asthmatic responses in an allergen-challenge study in patients with mild atopic asthma. Treatment with AMG157 moderately suppressed blood eosinophils over the course of the study and profoundly decreased sputum eosinophils after allergen challenge (Gauvreau et al., 2014). AMG157 is currently in Phase II studies in severe, uncontrolled asthma (NCT02054130).

Bertilimumab (Immune Pharma) is a fully human anti-eotaxin-1 mAb in development for asthma with Phase II trial results expected in 2016. Eotaxin-1 (CCL11) is a potent eosinophil chemoattractant that is

a major contributor to tissue eosinophilia. CCR3 is the main high affinity receptor for eotaxin-1, but it is promiscuous and can be activated by a wide range of chemokines. Although its expression was initially thought to be limited to eosinophils, CCR3 is now recognized to be more widely expressed (e.g., on basophils, mast cells, Th2 T cells). By binding to CCR3, eotaxins-1, 2 (CCL24) and 3 (CCL26) recruit eosinophils to sites of inflammation and activate them. Patients with acute asthma have elevated CCL11 plasma concentrations compared with patients with stable asthma (Lilly et al., 1999), and patients with asthma exhibit increased expression of CCL11 and CCL24 in their allergic lungs (Ying et al., 1999) and in their sputum (Yamada et al., 2000). Furthermore, patients with atopic asthma have increased CCR3 mRNA and protein expression. Increased expression of CCR3 is associated with AHR in patients with asthma (Ying et al., 1997). An anti-CCR3 mAb and small molecule CCR3 antagonists suppressed airway eosinophilia in models of allergic airway inflammation (Justice et al., 2003; Wegmann et al., 2007; Komai et al., 2010). However, the first clinical study of patients with asthma with eosinophilic inflammation with a small-molecule oral CCR3 antagonist, GW766994, achieved plasma drug concentrations consistent with 90% receptor occupancy, but it did not significantly decrease eosinophil counts in blood or sputum. This finding has called into question the potential for CCR3 antagonists to reverse established airway eosinophilia in asthmatics (Neighbour et al., 2014). Historically, several pharmaceutical companies have had programs to develop antagonists to CCR3 or its activating chemokine eotaxin, but relatively few of these are still active. Targeting of eotaxin-1 with bertilimumab has the potential for redundancy with other CCR3-activating chemokines, which is clearly a key issue for this approach. AXP-1275 (Axikin) and GW-766904 (GSK) are orally available, small-molecule inhibitors of CCR3 reported to be in Phase II development for asthma. Overall, the potential for eotaxin and CCR3 antagonists in the treatment of asthma looks very uncertain.

Fig. 1b illustrates all the therapeutic antibodies discussed here and their molecular targets and modes of action. The table captures their properties (antibody formats and affinities) and summarizes the effects of these therapeutic antibodies on eosinophil concentrations and their clinical efficacies in patients with asthma.

7. Future directions for *anti*-eosinophilic therapies

Development of new therapeutic antibodies targeting asthma and COPD with eosinophilic inflammation holds promise to provide much-needed help for patients. In addition, arising from the advent of these therapies, a number of areas are ripe for further exploration.

7.1. Biomarkers

As severe asthma is a heterogeneous disease with several inflammatory phenotypes (Gauthier et al., 2015), identifying biomarkers to select patients who are most likely to gain clinical benefit by monoclonal antibody therapies that target specific cytokines or inflammatory pathways is a useful strategy. Furthermore, pragmatic and easy-to-use sampling methods, such as blood tests, facilitate biomarker use. For example, methods for sampling the airways by induced sputum collection or endobronchial biopsies are invasive and require specialized equipment and trained personnel, often limiting their use to clinical research rather than clinical practice. To evaluate blood biomarkers predictive of eosinophilic inflammation in the airway, several studies have investigated the correlations of different blood biomarkers with eosinophilic inflammation in patients with asthma (Jia et al., 2012; Hastie et al., 2013; Wagener et al., 2016). Relationships between blood biomarkers and airway inflammation may vary, based on the patient populations studied, assays applied, time and method of sampling, and medications patients use. For example, some studies identified blood eosinophils as a poor predictor of eosinophilic inflammation in the airways (Hastie et al., 2013), while more recent studies suggest that blood eosinophils may be good predictors of eosinophilic inflammation of the airway in asthmatics (Wagener et al., 2016). Establishing response to therapy in clinical studies with patients stratified by biomarker high vs biomarker low ultimately determines the usefulness and predictive utility of biomarkers. To that end, elevated biomarkers such as IgE, circulating eosinophils, or serum periostin/dipeptidyl peptidase-4 have already been shown to predict improved treatment response to anti-IgE, anti-IL-5/anti-IL-5Rα, and anti-IL-13 therapeutic antibodies, respectively (Skiepko et al., 2014; Castro et al., 2014; Brightling et al., 2015a). However, none of these markers alone is a perfect predictor of treatment response, as they overlap with one another (Tran et al., 2016), and some biomarkers may work best in the context of certain clinical characteristics (e.g., more reversible airway disease, patient age at disease onset). They may also vary over time and with treatment (e.g., systemic corticosteroids) and, therefore, disease progression. As discussed above, eosinophil degranulation products may offer an alternative approach, but this has yet to be validated. Further, clinical research is required to improve the use and predictability of such markers and to identify new biomarkers that may be more predictive of molecular mechanisms in the airway, and consequently clinical outcomes.

7.2. Early intervention and intervention in patients with mild to moderate asthma

Children with severe asthma have persistent, steroid-resistant pulmonary eosinophilia associated with significant airway remodeling (Bossley et al., 2012). Therefore, intervention with eosinophil-targeting therapeutic strategies early in life may both provide immediate relief and have the potential to avoid irreversible structural changes in the airways that could limit treatment responses later in life. For patients with advanced disease, disease activity reflects damage as well as inflammation. Consequently, any therapeutic intervention has a substantial, potentially irreversible component to overcome. A similar argument could be made for the treatment of adults with mild to moderate asthma to prevent remodeling and accelerated decline in lung function. There is indeed precedent for the successful application of an "early intervention paradigm," which comes from studies in patients with rheumatoid disease. Patients with rheumatoid arthritis who were treated at disease inception with an aggressive combination of methotrexate and a TNF blocker responded to therapy rapidly, and a significantly greater percentage of patients achieved remission (Emery et al., 2012; Emery, 2014).

In addition, infrequent administration of these parenteral therapeutics may improve compliance in pediatric and adult patients with less severe disease. Furthermore, the acceptable safety profile of systemically administered therapeutic antibodies in adult patients provides support to expand clinical trials to pediatric patient populations and those patients with less severe disease. Interestingly, evidence in preclinical models has recently been published suggesting a role for eosinophils in adipose tissue homeostasis (Wu et al., 2011; Qiu et al., 2014) and in supporting plasma cell survival and promoting humoral responses through their interactions with B cells (Chu et al., 2011; Chu & Berek, 2012). However, no observations from clinical studies targeting eosinophils have been made yet to corroborate these findings. Although this discrepancy may be attributable to a difference between species, patients should be monitored appropriately in ongoing clinical trials.

7.3. Expansion of anti-eosinophilic therapies to other indications

The acceptable systemic safety profile of the *anti*-eosinophilic therapies discussed in this review may provide an upside for the treatment of comorbidities outside the lung, such as nasal polyposis or eczema, which are often associated with asthma. There is also opportunity to provide benefit for patients with eosinophilic esophagitis, chronic rhinosinusitis with polyposis, hypereosinophilic syndrome, Churg-Strauss syndrome, and eosinophilic gastrointestinal diseases, all of

which are characterized by eosinophilic inflammation and represent diseases in dire need of new treatment options. Clinical trials with anti-IL-5 and anti-IL-5Rα therapies are already underway in some of these indications.

8. Conclusions

For patients with severe asthma and COPD with eosinophilic inflammation, clinical symptoms are often poorly controlled despite the use of high-dosage inhaled and systemic corticosteroids. Monoclonal antibodies targeting cytokines and receptors implicated in the pathogenesis of eosinophilic inflammation have emerged as potent adjunct therapies providing additional benefits for these patients. The approvals of mepolizumab and reslizumab as add-on therapies for maintenance treatment of patients with severe asthma and the development of anti-IL-5R antibodies for patients with asthma and COPD with eosinophilic inflammation will provide additional treatment options for subgroups of patients who have an inadequate or incomplete response to corticosteroid therapy. Because of the specificity of these therapeutics, they are generally well-tolerated with limited off-target adverse effects (mainly injection-site reactions and limited immunogenicity). This safety profile could, eventually, allow their use in patients with less severe disease, pediatric patients, and other eosinophilic indications.

The advent of a range of antibodies targeting different mechanisms in asthma should enable progress toward the goal of personalized medicine, in which existing biomarkers aid clinicians in matching each biologic therapy to the patient population most likely to benefit from that treatment. Future directions for research should include discovery of new biomarkers, exploration of combination therapies that target multiple pathways, and recognition of the disease underlying pathologies in additional patient subpopulations who might benefit from novel treatments.

Conflict of interest statement

J Nixon, P Newbold, T Mustelin, and R Kolbeck are full-time employees of MedImmune LLC, a part of AstraZeneca, the manufacturer of benralizumab and tralokinumab, and a co-manufacturer of AMG157/MEDI-9929. GP Anderson has received speaker honoraria and served on advisory boards from companies whose compounds are discussed in this paper, including Novartis and AstraZeneca. In 2016 GP Anderson undertook a sabbatical secondment in Cambridge, UK, funded by AstraZeneca.

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